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FORMATION OF SULPHUR–SULPHUR BONDS BY THE NUCLEOPHILIC ATTACK ON SULPHENYL HALIDES AND THIOCYANATES

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Abstract. Aromatic sulphenyl chlorides were treated with sodium azide to prepare the corresponding sulphenyl azides in the presence of nitrene trapping agents, i.e. dimethyl sulphoxide, cyclohexene and norbornylene. No expected adduct was obtained but instead the corresponding disulphides were found in quantitative yields. Similar results were obtained when sulphenyl thiocyanate was treated with sodium azide. Disulphides did not either react with sodium azide or iodine azide. Sulphenylsodium salt also gave the corresponding disulphide in very high yield.

Sulphenyl azide (I) is to the author's knowledge, yet to be prepared. Benzenesulphinyl azide,[1] the first of its class, has been reported to give the corresponding nitrene. It seems of interest to study the reactions which could proceed through the intermediate formation of sulphenyl azide. If sulphenyl azide was actually produced and then lost its N to generate a sulphenyl nitrene (II), an aziridine formation would be expected in the presence of olefinic compounds, while in the presence of dimethyl sulphoxide sulphenyldimethylsulphoximine would be expected.

$$\underset{\text{(I)}}{RSN_3} \longrightarrow \underset{\text{(II)}}{RS\ddot{N}:} + N_2$$

Sodium azide was treated with *p*-toluenesulphenyl chloride in acetonitrile, in the presence of dimethyl sulphoxide at −30°C, nitrogen was evolved and dark red colouration was observed which gradually disappeared and nearly quantitative yield of *p*-toluenedisulphide was obtained. The substituted aromatic sulphenyl chlorides gave the corresponding disulphides (Table 1).

$$RSCl + NaN_3 \longrightarrow \tfrac{1}{2}\,RSSR + NaCl + \tfrac{1}{2}\,N_2$$

TABLE 1.

R	RSSR (% yield)	M.p.
C_6H_5	98	57–58°
p-CH_3—C_6H_4	90	45–46°
p-NO_2—C_6H_4	96	184–86°
m-NO_2—C_6H_4	95	195°
2,4-di-NO_2—C_6H_3	98	198–9°

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The reaction between the iodine azide and *p*-nitrobenzenesulphenylsodium in a mixture of acetonitrile and dimethyl sulphoxide was carried out, the red colouration of sodium salt (III) slowly discharged with the evolution of nitrogen gas. The product isolated from the reaction mixture was the corresponding disulphide in about 85% yield. Similar results were obtained when acetonitrile was used as solvent and norbornylene was used as sulphenyl azide trapping agent.

$$RSNa + IN_3 \longrightarrow \tfrac{1}{2}RSSR + NaI + 1\tfrac{1}{2}N_2$$

R=NO_2–C_6H_4 and CH_3–C_6H_4

The sulphenyl thiocyanates have been reported to resemble the sulphenyl halides,[2] as thiocyanogen is believed to be pseudohalogen. Reaction between *o*-nitrobenzenesulphenyl thiocyanate, and sodium azide was carried out under similar conditions, using the same solvents. Brisk evolution of nitrogen was observed alongwith formation of dark red colouration, which slowly discharged. The reaction mechanism could be postulated on the basis of these results. The initial attack of the azide ion on the sulphenyl chloride or thiocyanate gives the sulphenyl anion. The sulphenyl anion in turn reacts with Na^+ to give sodium salt (III) which gives the dark colouration in the reaction. The sodium salt could then react with the sulphenyl chloride or sulphenyl thiocyanate to give the corresponding disulphide, sodium chloride and sodium thiocyanate respectively.

$$RSX + \bar{N}_3 \longrightarrow XN_3 + \bar{RS}$$

$$\bar{RS} + \overset{+}{Na} \longrightarrow RSNa$$

$$RSNa + RSX \longrightarrow RSSR + NaX$$

X=Cl or SCN; R, =C_6H_5—, CH_3 — C_6H_4 —, $NO_2C_6H_4$—, and di-NO_2. C_6H_4—

The sulphur–sulphur bonds are subject to both homolytic and heterolytic cleavage.[3] Homolytic cleavage occurs by direct dissociation (eq 1) or as a result of attack of a free radical on one of the sulphur [4,5] (eq 2). Heterolytic cleavage can involve attack by a nucleophile[6] (eq 3) or assistance by electrophile (eq 4) or both[8] (eq 5).

$$RSSR \longrightarrow 2R\dot{S} \qquad (1)$$

$$\dot{R}\cdot + RSSR \longrightarrow \overset{\prime}{R}SR + R\dot{S} \qquad (2)$$

$$\bar{N}u + RSSR \longrightarrow RSNu + R\bar{S} \qquad (3)$$

$$\overset{+}{E} + RSSR \longrightarrow \overset{+}{R}SSR \qquad (4)$$

$$\overset{+}{R}S + RSE \longleftarrow \underset{|}{E}$$

$$\bar{N}u + \overset{+}{R}SSR \longrightarrow RSNu + RSE \qquad (5)$$

$$\underset{|}{E}$$

These results encouraged us to look at the reactions of disulphides with sodium azide and iodine azide; but all the efforts to prepare sulphenyl azide proved to be unsuccessful as the disulphide was recovered in quantitative yield from the reaction mixture, although the reaction time was prolonged up to one week, with stirring at room temperature in acetonitrile and dimethyl sulphoxide mixture. The temperature was also raised to refluxing (82°) but no change in results was observed.

The benzenesulphenyl azide has been reported to be stable at low temperature, therefore, attempts were made at 0–35°. Iodine azide was also used, keeping in view the work of Kice[3,8] as both the nucleophile and electrophile (eqs 4, 5) could be involved in the reaction; but results similar to those of the sodium azide were obtained.

It could be concluded in the light of these results that in the reactions between sodium azide or iodine azide with sulphenyl chloride or sulphenyl thiocyanate the nucleophilic attack converts the sulphenyl group into a better leaving group than the chloride or thiocyanate groups.

Experimental

Reaction of p-Toluenesulphenyl Chloride with Sodium Azide

A solution of *p*-toluenesulphenyl chloride (2.0 g) in acetonitrile (20 ml) was added dropwise with stirring, in 20 min to a suspension of sodium azide (20 g) in a mixture of acetonitrile (60 ml) and dimethyl sulphoxide (15 ml) at—35° (external bath temperature was –40°). Dark red colouration appeared soon after the addition of sulphenyl chloride. The mixture was stirred at –30° for ½ hr, during this time the colour disappeared with evolution of gas, leaving a yellow solution. The solvent was removed and residue crystallised from benezene and light petroleum (1:1) mixture. *p*-Toluenedisulphide (1.366 g, 90% yield, m.p. 45–46°) was obtained, spectral data resembled to the one reported in literature.

Reaction with Other Sulphenyl Chlorides

Benzenesulphenyl Chloride. Under the conditions stated above, benzenedisulphde, m.p. 57–58° was obtained in 98% yield.

m-*Nitrobenezenesulphenyl Chloride.* Under the conditions mentioned above *m*-nitrobenzenedisulphide (m p. 195°) was obtained in 95% yield.

p-*Nitrobenzenesulphenyl Chloride.* Undter the above mentioned conditions *p*-nitrobenzenedisulphide (m.p. 181°) was obtained in 96% yield.

2,4-*Dinitrobenzenesulphenyl Chloride.* Reaction carried under the the conditions mentioned above gave 2,4-dinitrobenzenedisulphide (m.p. 198–99°C) in 98% yield.

Reaction of p-*Toluenesulphenyl Chloride with Azide Ion*

p-Toluenesulphenyl chloride (4.0 g) in acetonitrile (20 ml) was added dropwise in 15 min, to a suspension of sodium azide (4.5 g) in acetonitrile (90 ml) at –35° (temperature of the bath was kept near –40°C) and allowed to stir for 10 min. To the dark red solution cyclohexene (20 ml) was added. The colour discharged in about 20 min after which the temperature was allowed to rise to that of room temperature. Chloroform (100 ml) was added, the reaction mixture was filtered, washed twice with water, dried and the solvent evaporated. The residue chromatographed on silica column and two fractions obtained. (1) *p*-Toluenedisulphide (2.327 g, 75% yield, m.p. 45–6°) IR fits the structure. (2) *p*-Toluenechlorocyclohexenesulphide. (1.214 g, 20% yield, m.p. 36–38°, lit m.p. 37–38°) IR spectrum fits the structure.

Reaction of o-*Nitrobenezenesulphenyl Thiocyanate with Azide Ion*

To a solution of *o*-nitrobenzenesulphenyl thiocyanate (0.424 g) in dry dimethyl sulphoxide (30 ml), sodium azide (0.65) was added at room temperature. Brisk evolution of nitrogen started as soon as sodium azide was added and the colour of reaction mixture became dark red. The reaction mixture was stirred for 1 hr, poured into cold water and precipitate filtered. *o*-Nitorobenzenedisulphide. was dried and crystallised from benzene (0.307 g, 100% yield, m.p. 195°).

Attempted Reaction of p-Nitrobenezendisulphide with Iodine Azide

p-Nitrobenzenedisulphide (1.5 g) was dissolved in chloroform (100 ml) and dimethyl sulphoxide (20 ml) and to this solution iodine azide (25 ml of 0.11 mole) was added dropwise at –35° with stirring. The temperature was allowed to rise to room temperature in about 3 hr and stirring was continued overnight. The reaction mixture was treated with water, organic layer separated, dried ($MgSO_4$) and *p*-nitrobenzenedisulphide was recovered in quantitative yield.

Reaction of p-Nitrobenzenesulphenylsodium with Iodine Azide

p-Nitrobenzenesulphenylsodium (1.7 g) was taken in acetonitrile (150 ml) and diemethylsulphoxide (10

ml). To the stirred solution iodine azide (15 ml, 11 mole) was added dropwise at −35°. The red colour of the sodium salt slowly discharged (about 1 hr). The reaction mixute was warmed up to room temperature and the solvent was evaporated under reduced pressure. The residue gave *p*-nitrobenzenedisulphide (1.271 g, 85% yield, m.p. 181).*

*When this reaction was carried out in the presence of norbornylene, no adduct of sulphenyl azide was obtained. p-Nitrobenzenedisulphide was obtained in 80% yield.

References

1. T.J. Maricich, J. Am. Chem. Soc., **90,** 7179 (1968).
2. E.E. Reid, *Organic Chemistry of Bivalent Sulfur* (Chemical Publishing, New York, 1958), vol. I, p. 272.
3. J.L.Kice, Acc. Chem. Research, **1,** 58 (1968) and reference cited therein.
4. W. A. Pryor, *Mechanism of Sulfur Reactions* (McGraw Hill, New York, 1962), p. 42.
5. (a) W.A. Pryor and H. Guard, J. Am. Chem. Soc., **86,** 1150 (1964). (b) W.A. Pryor and T.L. Pickering, *ibid.*, **84.**. 2705 (1962).
6. A. Fava, A. Ilceto and E. Camera, *ibid.*, **70,** 833, (1957).
7. R. E. Benesch and R. Benesch, *ibid.*, **80,** 1666, (1954).
8. J.L. Kice and E.H. Morkved, *ibid.*, **86,** 2270 (1964).

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ELECTROOXIDATION OF SILVER(I) NITRATE AND SILVER(I) PERCHLORATE IN THE PRESENCE OF 4,4′-DIMETHYL-2,2′-BIPYRIDYL; 2,2′-2″-TERPYRIDYL; 5,5′-DIBUTYL-2,2′-BIPYRIDYL AND 2,2′-BIQUINOLYL IN ACETONTRILE

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Abstract. Electrooxidation of silver(I) nitrate and silver(I) perchlorate in the presence of 4,4′-dimethyl-2,2′-bipyridyl and 2,2′,2″-terpyridyl enable the preparation and isolation of AgL_2 type complexes where silver is stabilised in Ag^{2+} state. Whereas the ligands 5,5′-dibuty 1-2,2′-bipyridyl and 2,2′-biquinolyl, though give indications where silver(I) is converted to silver(II) state, but the isolation of complexes was not successful. Particularly for 2,2′-biquinolyl, which due to its low solubility in acetonitrile created hindrance even in obtaining conclusive evidence in terms of coulometric determinations for silver(I) to silver(II) conversion.

The study on silver(I) salts in the presence of 2,2′bipyridyl (bipy),[1] using the basic technique exploited for the oxidation of organic compounds in nonaqueous solvents as reviewed by Weinberg and Weinberg,[2] prompted us to use ligands, 4,4′-dimethyl-2,2′-bipyridyl (dime-bipy), 2,2′,2″-terpyridyl (terpy), 5,5′-dibutyl–2,2′-bibpyridyl (dibut-bipy), and 2,2′-biquinolyl (biqu), capable of chelating satisfactorily with silver(I), by anodic polarography leading to controlled potential electrolysis corresponding to specific oxidation steps. Since the anodic range available is about 3 V vs S.C.E., the complexation with suitable ligands would permit oxidation of transition metal ions depending upon β and β_2 values for respective systems. The present work is restricted only to silver(I) salts in an effort to collect data for the metal with various ligands capable of π-bonding.

Experimental

The apparatus used for both the polarographic and controlled potential coulometric studies was basically the same as described earlier and comprised a potentiostat based on a design described by Hickling,[3] a reference voltage scanner capable of scanning between two voltages in the range + 5.0 to – 5.0 V in either direction at rates of scan varying between 1–1.5 mV/min, a potentiometric recorder with sensitivity of 1 mV, 10 mV and 100 mV for a full scale deflection alongwith chart speeds varying from 0.6 to 600 in/hr, and one of the two cells depending upon the mode of operation. Single compartment cell was used for polarographic studies and three compartment cell for controlled potential electrolysis. Rotating platinum microelectrode (R.P.E.) as described by Nernst and Merriam[4] was used for polarography. Tetra-n-butylammonium perchlorate (TBAP) was prepared[5,6] for use as a supporting electrolyte in 0.1M in acetonitrile which was dried to only 50 p.p.m. of water content.

Studies were carried out in a dry box filled with oxygen-free nitrogen maintained at R.H. 5–8% at $20 \pm 1°$, since the acetonitrile solvent was capable of absorbing about 56 p.p.m. water in 3 hr, the normal time required for each set of experiment.

It is well known [8,9] that the ohmic drop between the working electrode and reference electrode in high resistance system is not completely compensated for in three electrode resistance-compensating potentiostat, even though the current flowing in the reference cell is zero. Although theoretical calculations[8,10] have been made for the ohmic drop occurring in the vicinity of a D.M.E. The problem is much more complicated when a R.P.E. is employed and theoretical calculation is not possible. To evalute the magnitude of this effect, polarograms of silver(I) nitrate were obtained where the distance between the tip of the reference electrode and R.P.E. was varied, while the other parameters were kept constant. This attempt led to the conclusion that if the distance between reference electrode tip and R.P.E. is kept from 0.5 to 1.0 mm approximately, the ohmic drop correction was negligible under the particular cell conditions used.

Determination of Silver(II). A novel method was developed for the determination of small amounts of silver(II). The method described by Dutta[7] involving the oxidation of iodide by silver(II) was found to be unsuitable because silver(II) complexes with polypyridyls were found to give an incomplete reaction with potassium iodide solution in the cold. It was thought that this was due to the adsorption of iodine onto the surface of the crystals and that this prevented further reaction. This difficulty was apparently overcome by refluxing the solution, however, using a water condenser, some iodine was lost and further it was difficult to remove all traces of iodine from the condenser. The method described here used bromide ion as the reductant, the liberated bromine being readily removed from the reaction mixture.

The apparatus used for silver(II) determination is shown in Fig. 1 and consists of a 50 ml pear-shaped distillation flask fitted with a nitrogen inlet and reflux condenser leading to a trap 'A' containing potassium iodide solution. A second safety trap contained a solution of potassium iodide to which was added a few drops of strach solution.

An accurately weighed amount of silver(II) complex (usually about 50 mg) was introduced into the pear-shaped flask together with about 10 ml de-

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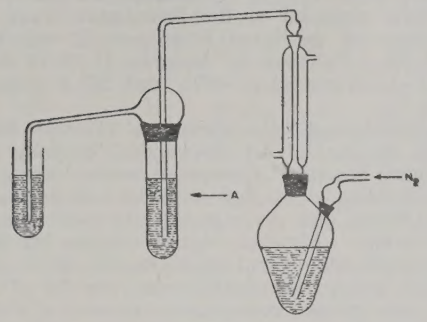

Fig. 1

oxygenated water and about 100 mg analar grade potassium bromide. The oxygen-free nitrogen supply was switched on and the solution was heated to boiling using a microburner and refluxed for about 5 min. At this stage most of the bromine had passed over into the iodide trap, the last traces of bromine in the reaction vessel were removed by increasing the nitrogen flow and switching off the water supply to the condenser until steam just began to condense into the delivery tube. With the water supply restored, the nitrogen flow was continued for another minute.

The iodine solution was transferred to a 100 ml conical flask together with washings from the delivery arm and titrated against a standardised 0.01N solution of thiosulphate contained in a weighed burette using starch solution as indicator. A known weight of Analar grade potassium bromate was placed in the reaction vessel to which was added an excess of analar KBr and 10 ml 0.5N sulphuric acid. The bromine thus produced was transferred to the iodide trap by the producere described above and the resulting iodine titrated by weight against the 0.01N thiosulphate solution.

The use of 0.01N solution of thiosulphate together with the weight titration technique allowed small quantities of silver(II) complexes to be accurately analysed.

Isolation and Analysis of 4,4′-*Dimethyl*–2,2′-*bipyridyl Complex.* After each electrolysis for silver(I) salts in the presence of dime-bipy, the anolyte was immediately transferred to a round-bottom flask and the solvent was removed under vacuum at room temperature. The residue consisted of excess of ligand, (TBAP) and the silver(II) complex. Impurities were successfully removed by selecting dry ethyl acetate for washing, whereas every unwanted species, except the complex was soluble. About seven washings were required to obtain a pure product. The product was dried in vacuum for 2 hr at 30°C and stored in desiccator over phosphorous pentoxide. The technique[1] of pushing out of the complex by adding silver(I) salt in anolyte was successful in this case as well. But evaporation method was preferred, since the technique gave better yield. The C, H, N and Ag^{2+} analysis of the product obtained from silver(I) nitrate and silver(I) perchlorate are given as follows alongwith the theoretical values for $[Ag(dime\text{-}bipy)_2]NO_3ClO_4$ and $[Ag(dime\text{-}bipy)_2](ClO_4)_2$.

		C(%)	H(%)	N(%)	Ag^{2+}(%)
$Ag(dime\text{-}bipy)_2$ NO_3ClO_4	Calcd:	45.19	3.79	10.98	16.91
	Found:	45.18	3.77	9.88	16.6
$Ag(dime\text{-}bipy)_2$ $(ClO_4)_2$	Calcd:	42.69	3.58	8.30	15.97
	Found:	42.29	3.59	8.19	15.6

The products were isolated from the anolyte (of silver(I) salts in the presence of bipy) in the same way as used for silver(II)/dime-bipy, except that the solvent used for washing was chloroform instead of ethyl acetate. The process of adding silver(I) salt in anolyte for pushing out silver(II) complex were not successful for terpy which were exploited quite successfully for silver(II)/bipy[1] and silver(II) dime-bipy as mentioned earlier. The C, H, N and Ag^{2+} analysis of the products obtained from silver(I) nitrate and silver(I) perchlorate in the presence of terpy alongwith the theoretical values are given as follows:

		C(%)	H(%)	N(%)	Ag^{2+}(%)
$Ag(terpy)_2$ $(ClO_4)_2$	Calcd:	46.59	2.87	10.87	13.95
	Found:	45.8	2.9	10.8	13.7
$Ag(terpy)_2$ $ClO_4.NO_3$	Calcd:	48.96	3.01	13.32	14.66
	Found:	46.6	3.3	10.8	13.5

Results and Discussion

Anodic Polarography of Polypridyls. Since polarograms for silver(I) nitrate and silver(I) perchlorate were obtained[1] it was concluded[1] that silver(I) is not oxidised to silver(II) in the absence of ligands, The anodic polarography of these polypridyls alone was therefore, carried out first, to make sure that ligands themselves do not get oxidised within the anodic range, and if so, to determine the extent of the interference which might be expected. A small wave was observed at a half wave potential ($E\,\frac{1}{2}$) of 2.04 V for dime-bipy, the limiting current being about 0.3 μA. Several polarograms were obtained at different concentrations of the ligand (up to 10 mM and it ws found that the limiting current was independent of concentration for the particular electrode, as has already been noted in the case of 2,2′-bipyridyl.[1] The anodic polarography of dibut-bipy and terpy gave somewhat smaller limiting currents (i_L), i.e. about 0.2 μA; the $E\,\frac{1}{2}$ were 2.0 V and 1.9 V respectivley. Again the limiting currents were found to be independent of concentration. But, the polarographic study of biqu was limited to solutions having concentrations in the range1–2 mM due to the low solubility of this ligand in acetonitrile. The limiting current for biqu was found to be only 0.1 μA and half-wave potential was about 1.9 V; a value similar to terpy.

The observation of small limiting current for all these ligands remain constant over a wide range of conentration. The use of graphite electrode[11] might solve the mystery of this i_L which could possibly be due to platinum-to-ligand complexation. Nothing, however, has been recorded in the literature regarding this phenomenon.

Anodic Polarography of Silver(I) Salts in the Presence of Polypyridyls. The main purpose of the polarographic study was to determine the anodic potentials required for the electrooxidation of silver(I) complexes as a preliminary to preparative scale electrolyses.

Silver(I) nitrate and silver(I) perchlorate in the presence of polypyridyls using 0.1 M TBAP as supporting electrolyte, and scanning at 0.4 V/min. gives i_L varying with conentrations (Table 1).

Two well-defined waves were observed in case of silver nitrate–polypyridlyl systerms; for the silver perchlorate–polypridyl systems the second wave is usually complicated by a maximum which is followed by a marked drop in current. Because of this effect, values of i_L for second wave is not quoted in Table 1, and half-waves are only approximate. Values for the i_L of the first wave for these systems are again small compared to the values expected for a one-electron change based on the total metal present. In the case of silver(I) nitrate–bipy[1] system, this effect was explained in terms of the involvement of the intermediate species, Ag (bipy)$^+$ Unfortunately, stability constant data are not available to enable similar calculations to be made of the intermediate complexes associated with the silver(I)/ polypyridyl systems. However, it is found in all cases, that the i_L is reduced to low values when ligand is in excess in qualitative agreement with the dependence of limiting current on the concentra tion of an intermediate species. In the case of silver(I) salt–biquinolyl system, the values of the i_L appear to be much smaller than the corresponding values for bipy system; this may be due to a higher ratio of the successive stability sonstant (K_2/K_1) for 2,2′ biquionlyl system which would lead to a reduction in the concentration of the intermediate species. The half-wave potential of the first anodic wave with the nature of the ligand as follows: 2,2′–biquinolyl (1.74 V), 5,5′-dibutyl-2,2′ bipyridyl (1.42 V)' 4,4′-di methyl-2,2′-bipyridyl (1.40V), and 2,2′,2″-terpyridyl (1.30 V).

TABLE 1. ANDOIC POLAROGRAPHY OF SILVER(I) SALTS IN THE PRESENCE OF LIGANDS.

	C_M^* (mM)	C_L^* (mM)	i_L (μA)	$E_{\frac{1}{2}}$(I) (V)	$E_{\frac{1}{2}}$(II) (V)	Ligand
Silver perchlorate	2.85	6.09	0.9	1.40	2.0	Dime bipy
	2.85	14.38	0.3	1.41	1.9	
	3.69	6.09	0.6	1.40	2.0	
Silver nitrate	1.62	1.70	0.4	1.31	1.91	Terpy
	1.11	20.20	0.1	—	1.93	
	4.00	1.70	0.7	1.29	1.95	
Silver perchlorate	0.92	1.74	0.2	1.30	2.0	Terpy
	0.92	15.33	0.0	—	1.9	
	3.56	6.12	0.4	1.30	1.9	
Silver perchlorate	3.00	10.04	0.2	1.42	2.05	Dibut-bipy
	3.00	19.87	0.4	1.41	2.03	
	3.00	47.35	0.1	1.43	2.04	
Silver nitrate	0.52	2.01	0.3	1.76	2.15	Biquinolyl
	3.01	3.00	1.2	1.76	2.02	
	2.02	4.00	0.7	1.75	2.05	

* C_M = Total concn of metal salt * C_L = Total concn of ligand

Electrolysis of Silver Nitrate and Silver perchlorate in the Presence of 4,4′-Dimethyl-2,2′-dipyridyl at its Half-wave Potential. Controlled potential electrolysis corresponding to the first wave were carried out for silver nitrate and silver perchlorate in the presence of dime-bipy. Metal salt–ligand ratio was 1:2, which enabled the smooth coulometric determinations as no product covered the working electrode' In all cases there was almost one-electron oxidation (Table 2) indicating that the first polarographic wave corresponds to the oxidation of silver(I) to silver(II).

The C, H, N and Ag^{2+} analysis for the dark red crystals dec. at 200°) obtained from silver(I) nitrate corresponds to the theoretical anaylsis for the compound Ag (dime-bipy)$_2$ NO_3CLO_4. The spectrum of the above product showed in ionic nitrate band at 1387 cm^{-1} and a strong, broad band at 1092 cm^{-1} corresponding to ionic perchlorate, indicating the presence of nitrate and perchlorate anions. The diffeuse reflectance spectrum gave a single absorption band at 19,417 cm^{-1} which probably is associated with the d–d transition of the d^9 configuration. The mass spectrum gave a maximum mass number of 184.1002 corresponding to the mass of the 4,4′-dimethyl-2,2′-bipyridyl molecule. It appears that the complex is decomposed under the conditions used to obtain the mass spectrum (ion source temperature 220°, electron energy 70 eV). However, the result is useful in confirming that the ligand is not oxidised.

The C, H. N and Ag^{2+} analysis for the red crystals (m.p. 162–162°) obtained from silver(I) perchlorate corresponds to the theoretical values for the compound Ag(dime-bipy)$_2$(ClO_4)$_2$. The IR spectrum of the product showed a strong broad band at 1090 cm^{-1} indicating the presence of an ionic perchlorate group. The diffuse reflectance spectrum gave a single abosorption band at somewhat higher frequency (20,576 cm^{-1}) compared to the mixed nitrate–perchlorate complex. The mass spectrum gave again, only the number corresponding to ligand alone.

Electrolysis of Silver Nitrate and Silver Perchlorate in the Presence of 2,2′,2′-Terpyridyl at its Half-Wave Potential. Controlled potential electrolysis corresponding to the first wave were carried out for silver nitrate and silver perchlorate in the presence of terpy. Preparation and coulometric determinations were obtained simultaneously where metal salt–ligand ratio was 1:2. This ratio was sufficient for keeping the working electrode clean throughout the electrolysis. Coulometric determinations for silver nitrate as well as silver perchlorate gave convincingly one eletron change for silver(I) to silver(II) process (Table 3).

The C, H, N, and Ag^{2+} analysis for the product obtained from silver nitrate–terpy system was anticipated to correspond to the complex having 1:1 terpy to silver ratio as reported by Morgan and Burstall[12] who claim to have isolated[Ag(terpy)]NO_3. Further, comparison with the product obtained from the oxidation of silver(I) nitrate in the presence of bipy

and dime-bipy, a mixed anion complex was expected. However, the analytical data are consistent with the formulation $Ag(terpy)_2(ClO_4)_2$, The IR spectrum of the product gave neither an ionic nitrate band in the region of 1380 cm^{-1}, nor a covalent nitrate band in the region of 125 cm^{-1}. Strong bands in the region of 1000–1200 cm^{-1} and at 920 cm^{-1} were also absent, These bands are characteristic of coordinated perchlorate group as described by Wickenden and Krause[13] but a strong and broad band in the region of 1090 cm^{-1} was observed, indicating the presence of ionic perchlorate group.

The C, H, N, and Ag^{2+} analysis for silver(II) complex obtained from silver(I) perchlorate–terpy system is very close to the analysis of the complex obtained from silver(I) nitrate–terpy system, i.e. for the product having molecular formula $Ag(terpy)_2(ClO_4)_2$. The IR spectrum also supports this formulation, as a broad and strong band in the region of 1090 cm^{-1} was observed, which is charactertistic of ionic perchlorate. Disappointingly, mass spectrum gave again the maximum mass of 233.0953 corresponding to the mass of terpy molecule only, as has been observed for dime-bipy complexes.

Although the products obtained from silver nitrate and silver perchlorate gave the same analysis, there were differences in physical properties of the two complexes. For example, the product obtained from silver nitrate–terpy solutions gave dark-red plate-shaped crystals (m.p. 216–220°), whereas the silver perchlorate–terpy solutions gave dark–red needle-shaped crystals m.p. 208–215°C). The diffuse reflectance spectra showed a single absorption band at about 19,000 cm^{-1} for the product obtained from silver nitrate compared to a band at 18,200 cm^{-1} for the complex prepared from silver perchlorate. These data indicate possible existence of different isomers.

The complex bis(terpy)silver(II) diperchlorate suggests the possibility of six coordinated complex formation, whereas terpy acts as a tridentate ligand but structural data would be required to support the structure. It may, however, be noted that a three-dimensional X-ray analysis of silver(II) dipicholinate monohydrate [14] has shown it to have a distorted octahedral structure in which each ligand is tridentate.

Electrolysis of Silver Perchlorate in the Presence of Dibut-Bipy at its first Half-wave potential. Preparative

TABLE 2. COULOMETRY OF SILVER(I) SALTS–DIME-BIPY SOLUTIONS.

Wt. of silver salt (g)	Potential applied (V)	Initial current (mA)	Electrolysis time (min)	Current used (Coulomb)	Molecular ratios	Electron/mole
$AgClO_4$					Ligand: $AgClO_4$	
0·0253	1·38	2·97	92	12·140	2:1	1·03
0·0507	1·35	40	28	22·930	2:1	0·97
$AgNO_3$					Ligand:$AgNO_3$	
0·0368	1·46	80	20	19·568	2:1	0·94
0·0365	1·50	90	15	19·780	2:1	0·95

TABLE 3. COULOMETRY OF SILVER(I) SALTS–TERPY SOLUTIONS.

Wt of Silver salts (g)	Potential applied (V)	Initial current (mA)	Electrolysis time (min)	Current used (Coluomb)	Molecular ratio of Teryp: Silver Salt	Electron/mole
$AgC1O_4$					Terpy:AgC104	
0·0104	1·20	2·25	60	4·830	2:1	1·00
0·0452	1·25	22	40	21·729	2:1	1·03
$AgNO_3$					Terpy:AgNO3	
0·0330	1·30	18·5	25	17·650	2:1	0·94
0·0322	1·35	22	21	19·946	2:1	1·06

TABLE 4 COULOMETRY OF SILVER(I) PERCHLORATE/DIBUT–BIPY SOLUTIONS.

Wt of $AgC1O_4$ (g)	Potential applied (V)	Initial current (mA)	Electrolysis time (min)	Current used (Coloumb)	Molecular ratio of Ligand:$AgClO_4$	Electron/mole
0·0311	1·4	8·3	96	16·500	3:1	1·14
0·0598	1·5	40	32	27·340	3:1	0·98

TABLE 5. SOLVENTS USED FOR ISOLATION OF DIBUT-BIPY SILVER(II) COMPLEX.

Solvent	Observation
Acetone	Completely soluable
Ethyl acetate	Completely soluble
Chloroform	Immediately dissolved
Ether	Colour of the product fades graduallay
Carbon tetrachloride	Decolourised
Nitromethane	Immediately decolourised
Methanol	Immediately decolourised
Benzene	Reddish brown viscous liquid settled down at the bottom of the test tube, stirred and decanted benzene. Repeated this treatment four times. Reddish brown liquid obtained which decolourised on drying under vacuum.

scale electrolyses were carried out at a fixed potential (in the range 1.4–1.5 V) corresponding to the first wave of the silver (I)perchlorate 5,5′-dibut-bipyl system with 0.1M tetra-n-butylammonium perchlorate as supporting electrolyte. Table 4 gives details of the electrolyses and the calculated number of electrons involved per molecule in the electrode reaction. As in all other silver(I) polypyridyl systems, the results confirmed that a one-electron oxidation process occurred at potentials corresponding to the first polarographic wave.

Repeated attempts to isolate the product obtained from these electrolyses were unsuccessful. The oxidised solution was dark red-brown charactertistic of silver(II)-polypyridyl complexes. A portion of this solution was treated with silver(I) salt but no silver(II) complex was precipitated, the colour of the anolyte remaining unaltered. Next, the solvent was removed under vacuum from the remainder of the solution until a viscous reddish brown residue containing an excess of supporting electrolyte and excess of 5,5′-dibutyl-2,2′-bipyridyl remained in the solution. The reddish brown residue gave iodine with aqueous potassium iodide solution indicating presence of silver(II). To isolate the product from the residue, a variety of solvents were used but without success. Table 5 gives a summary of the techniques used.

Electrolysis of Silver Nitrate in the Presence of 2,2′-biquinolyl at its Half-wave Potential. Controlled potential electrolysis of silver (I)nitrate solutions in the presence of 2,2′-biquinolyl using 0.1M TBAP as the supporting electrolyte was attempted. The potential used was 1.74 V which corresponded to the half-wave potential of the first anodic wave. However, it was found that, for this system, the initial current rapidly fell to very low levels apparently due to a deposit which formed on the electrode surface. Previous difficulties of this nature were overcome by the addition of an excess of ligand which prevented the deposition of the product on to the electrode surface. Unfortunately, for the present system, the low solubility of the ligand in acetonitrile did not permit this technique to be applied and coulometric determinations therefore, were not possible.

References

1. M.A.A. Usmani and D.B. Scaife, Pakistan J. Sci. Ind. Res., **18,** 224 (1975).
2. N.L. Weinberg and H.R. Weinberg, Chem. Rev., 449 (1968).
3. A Hickling, Electrochim. Acta, **5,** 161 (1961).
4. W. Nernst and E.S. Merriam, Z. Physik. Chem., **53,** 235 (1905).
5. W.F. Luder, P.B. Kraus, C.A. Kraus and R.M. Fuoss, J. Am. Chem. Soc., **58,** 255 (1936).
6. H.P. Schroer, F. Geuther and H.H. Horhold, Z. Chem., **8,** 309 (1968).
7. R.L. Dutta, J. Indian Chem. Soc., **32,** 95 (1955).
8. W.B. Schaap and P.S. Mckinney, Anal. Chem., **36,** 29 (1964).
9. S. Barnart, J. Electrochem. Soc., **108,** 102 (1961).
10. L. Nemec, J. Electroanal. Chem., **108,** 102 (1964).
11. S.S. Lord Jr. and L.B. Rogers, Anal. Chem., **26,** 284 (1954).
12. G.T. Morgan and F.H. Burstall, J. Chem. Soc., 1649 (1937).
13. A.E. Wickenden and R. A. Krause, Inorg. Chem. **4,** 404 (1965).
14. M.G.B. Drew, G.W.A. Fowles, R.W. Mathews and R.A. Watton, J. Am. Chem., Soc., **91,** 7769 (1969).

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SHELF-LIFE AND STABILITY STUDIES OF HEXAMMINECOBALT(III) TRICARBONATOCOBALTAE(III)

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Abstract. Hexamminecobalt(III) tricarbonatocobaltate(III) as titrant keeps its factor fairly constant over a reasonable period. Hydrochloric acid from 1 to 8N, sulphuric acid from 0.5 to 10.0N, acetic acid from 10 to 80 and 2 to 10% and perchloric acid have proved quite suitable media for redox studies with this titrant.

Hexamminecobalt(III) tricarbonatocobaltate(III) $[Co(NH_3)_6Co(CO_3)_3]$, relatively a newer redox titrant, has been successfully used in these Laboratories for the determination of various inorganic and organic compounds.[1-5] Its redox potential, in various acid media, being reasonably high,[6] lends importance for its use as a potential titrant in volumetric analysis. Whereas studies on utilizing its high redox potential in volumetric measurements for various determinations are being carried out by us, we thought it necessary to investigate about its shelf-life and suitability of the media of hydrochloric, sulphuric, acetic and perchloric acids. The media proved to be quite suited to our previous investigations.[1-5]

Experimental

Reagents. The solution of hexamminecobalt(III) tricarbonatocobaltate(III) was prepared and its normality checked according to previously described method.[1]

Hydrochloric, sulphuric, acetic and perchloric acids were of analytical grade (Merck), and the other reagents were either of A.R. or equivalent purity.

Apparatus. A pH meter (Pye, Cambridge, England) with saturated calomel as reference and platinum as indicator electrodes was used. A grade, officially calibrated, glassware was used throughout these investigations.

Procedure. An aliquot (5 ml) of $Co(NH_3)_6$-$Co(CO_3)_3$ solution was measured out by a 5-ml burette, calibrated at 0.01 ml intervals, in a 250-ml beaker. Water and the required acid were added to make the total vlume to 50 ml which was finally of required strength with respect to the particular acid. The contents of the reaction vessel were stirred with a magnetic stirrer and the potential reading was recorded when the needle of the potentiometer became stable. Successive potential readings were recorded at regular intervals. The whole procedure was repeated for the acids and their respective required concentrations.

For studying the shelf-life of Co(III) solution a standardised solution was divided into two portions, each one of them kept in a stoppered bottle, one in the dark and the other on the laboratory bench. The normality of these test solutions was checked after regular intervals according to the method already described.[1]

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Results and Discussion

Hydrochloric acid (1–8N) is a very suitable medium and it has almost no effect on the reduction of Co(III). Although the strength of Co(III) solution starts decreasing in 8N HCl after about 20 min inverals yet the medium is quite suited to many determinations. Sulphuric acid (0.5–10.0N), acetic acid (10–80%) proved to be quite suitable media for oxdiation studies of various substances with Co(III) as these acids like HCl have no tendency of reducing the titrant (Table 1).

TABLE 1. POTENTIALS (mV) AGAINST TIME (min), FOR VARIOUS CONCENTRATIONS OF HYDROCHLORIC, SULPHURIC, ACETIC AND PERCHLORIC ACIDS, IN A TOTAL VOLUME OF 50 ml CONTAINING 5 ml OF 00.0046N $Co(NH_3)_6(CO_3)_3$ SOLUTION.

Acid	Time (min)	Potential (mV)
Hcl	0	1095
1N	20	1095
	40	1090
	60	1085
	80	1080
	90	1075
	130	1075
3N	0	1045
	20	1045
	40	1040
	60	1040
	80	1035
	90	1035
5N	0	1020
	10	1020
	20	1020
	40	1015
	60	1015
	80	1010
	90	1010
8N	0	940
	10	920
	20	905
	50	885
	80	880
	90	880

(Table 1 continued)

H_2SO_4		
0.5N	0	1400
	10	1400
	30	1400
	50	1395
	70	1390
	90	1380
2N	0	1400
	10	1400
	30	1400
	50	1395
	70	1395
	90	1390
6N	0	1400
	10	1400
	30	1400
	40	1400
	80	1395
	90	1395
8N	0	1400
	20	1400
	60	1400
	70	1395
	90	1395
CH_3COOH		
	0	1400
10%	5	1400
	15	1400
	30	1395
	50	1395
	80	1390
	160	1385
30%	0	1395
	30	1395
	50	1398
	90	1385
	120	1380
	140	1370
		1365
50%	0	1390
	5	1390
	20	1390
	50	1385
	80	1385
	120	1380
80%	0	1380
	10	1380
	50	1380
	80	1275
	30	1370
H_2SO_4		
	0	1400
10N	20	1400
	70	1400
	90	1395
HCl		
	0	540
2%	5	700
	15	630
	40	590
	60	580
	90	590
4%	0	885
	15	800
	35	630
	65	580
	120	515
$HClO_4$		
	0	900
8%	15	890
	40	800
	70	552
	90	505
	130	490
10%	0	930
	20	820
	30	805
	35	770
	65	760
	90	740
	125	500

(Continued)

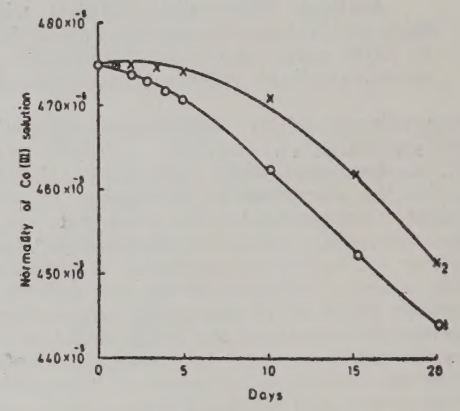

Fig. 1. Normality of Co(III) solution: (1) kept in light, and (2) kept in dark.

Water in the presence of perchloric acid reduces Co(III) and with increasing strength of the acid the rate of reduction is increased. When compared with other media like those of hydrochloric, sulphuric and acetic acids this medium (2–10% perchloric acid) stands relatively less acceptable for the purpose of its being used as a medium. Anyhow the reduction of the titrant, by water in the presence of various strengths of perchloric acid, is not as abrupt as to render it absolutely unfit for its use in oxidation studies.

Regarding shelf-life of the titrant it is evident that the solution kept in the dark is relatively more stable than the solution kept in the normal surroundings (Fig. 1). The change in normalities of both the lots is not so fast. After about five days the normalities start decreasing at quite a rapid rate. Within five days the slow rate of decrease of the strength of the titrant proves it to be reasonably suitable for determinations based on redox measurements.

References

1. M. Hanif, M. Saleem, Z. Sheikh and J. Zyka, Mikrochim. Acta, 625 (1976 II).
2. M. Hanif, M. Saleem and Z. Sheikh, Pakistan J. Sci. Ind. Res., **17,** 91 (1974).
3. M. Hanif, J.I. Toor and S.H. Qureshi, Pakistan J. Sci. Ind. Res., **17l,** 14 (1974).
4. M. Hanif and M. A. Shaida, Pakistan J. Sci. Res., **25,** (1973).
5. M. Hanif and M. Javaid, Pakistan J. Sci. Res., **25,** 4 (1973).
6. M. Hanif, M. Saeed, M. Z. Iqbal and J. Zyka, **19,** (1976).

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CONSTRUCTION OF MOLECULAR ORBITAL SEQUENCE OF SILVER(II)–BIS–TRIPYRIDYL SYSTEM

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Abstract. Molecular orbital sequence of silver (II)-bistripyridyl system has been determined on the basis of symmetry characters of the molecule. Effort has been made to explain the symmetrical octahedral structure.

Only a few Ag(II) complexes have been reported so far. The stability of these complexes depends amongst other things on the type of ligand. Tripyridyl provides one such ligand which is known to have an extended π-bonding system.[1] Ag(II) bistripyridyl has been prepared and its IR spectrum repoted. The IR spectrum indicates all coordinating nitrogens to be equivalent,[1] and hence the molecule can be considered to have cubic symmetry and O_h point group. An attempt has been made to determine the molecular orbital sequence of the system based on group symmetry properties.

The σ-System of Metal Ion. The following reducible representation was worked out using position functions for σ -bonds.

	E	$8C_3$	$6C_2$	$6C_4$	$3C_2$	i	$6S_4$	$8S_6$	$3\sigma_h$	$6\sigma_r$
$\Gamma\sigma$	6	0	0	2	2	0	0	0	4	2

The reducible representation can be broken down to irreducible representation using the relation.

$$n_i = \frac{1}{h} \Sigma_R \chi_r (R) \chi_i (R)$$

where n_i is number of times the i th irreducible representation appears in the reducible representation, h is the order of the group, χ_r (R) the character of matrix corresponding to operation R in reducible representation and χ_i (R) is the same in irreducible representation.

Using character table of O_h group $\Gamma\sigma$ was reduced to the following,

$$\Gamma\sigma = a_{1g} + e_g + t_{1u}.$$

It can be shown that a_{1g} transforms like S orbits, e_g like dz^2 and dx^2-y^2 and t_{1u} like px, py and pz orbitals.[2]

Hybrid Orbitals on Metal Ion. Since $\Gamma\sigma = a_{1g} + e_g + t_{1u}$ as shown earlier, the required orbitals resulting from the hybridization of these orbitals should be

$$\Psi_i = a_i S + b_i p_x + c_i p_y + d_i p_z + e_i d_{z^2} + f_i d_{x^2-y^2}$$

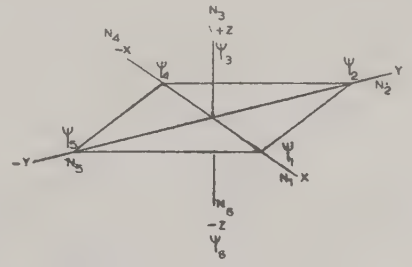

Fig. 1

Considering Fig. 1, it is evident that
Ψ_1 and Ψ_4 involves only S, p_x, dz^2, dx^2-y^2,
Ψ_2 and Ψ_5 involves only S, p_y, dz^2, dx^2-y^2,
Ψ_3 and Ψ_6 involves only S, P_z, and dz^2, hence the following relations can be derived:

$$a_1 = a_2 = a_3 = a_4 = a_5 = a_6 = A$$
$$b_3 = b_6 = b_2 = b_5 = 0$$
$$c_3 = c_6 = c_1 = c_4 = 0$$
$$d_2 = d_5 = d_1 = d_4 = 0$$
$$b_1 = -b_4,\quad c_2 = -c_5,\quad d_3 = -d_6 \Big\} = B, \text{ as } p_x, p_y \text{ and } p_z \text{ are qeuivalent orbitals}$$
$$e_3 = e_6 = c$$
$$e_2 = e_5 = e_1 = e_4 = -1/2c$$
$$f_3 = f_6 = 0$$
$$f_2 = f_5 = -f_1 = -f_4 = D$$

The hybrid orbits using the coefficients derived above can be written as:

$$\Psi_1 = As + Bp_x - 1/2\, Cdz^2 + Ddx^2y - ^2$$
$$\Psi_4 = As - Bp_x - 1/2\, Cdz^2 + Ddx^2y - ^2$$
$$\Psi_2 = As + Bp_y - 1/2\, Cdz^2 - Ddx^2y - ^2$$
$$\Psi_5 = As + Bp_y - 1/2\, Cdz^2 - Ddx^2y - ^2$$
$$\Psi_3 = As + Bpz + Cdz^2$$
$$\Psi_6 = As - Bpz + Cdz^2$$

Since these orbitals must be orthogonal and normalized, the mixing coefficients can be calculated to be the following.[3]

$A = 1/\sqrt{6}$, $B = 1/\sqrt{2}$, $C = 1/\sqrt{3}$, and $D = \frac{1}{2}$

The Ligand σ Orbital. Since we have

$\Gamma\sigma = a1g + eg + t1u$

suitable orbits from N-atoms of ligands that would transform like these irreducible representations should be constructed. If σi represents *ith* nitrogen atom we have the following set of orbitals.[4]

$$\varphi_1 = \frac{1}{\sqrt{6}}(\sigma_2 + \sigma_2 + \sigma_3 + \sigma_4 + \sigma_5 + \sigma_6) \quad a_{1g}$$

$$\left.\begin{aligned} \varphi_2 &= \frac{1}{\sqrt{2}}(\sigma_1\sigma_{-4}) \\ \varphi_3 &= \frac{1}{\sqrt{2}}(\sigma_2 - \sigma_5) \\ \varphi_4 &= \frac{1}{\sqrt{2}}(\sigma_3 - \sigma_6) \end{aligned}\right\} \quad \ldots\ \ldots\ \ldots\ t_{1u}$$

$$\left.\begin{aligned} \varphi_5 &= \frac{1}{\sqrt{2}}(\sigma_1 - \sigma_2 + \sigma_4 - \sigma_5) \\ \varphi_6 &= \frac{1}{2\sqrt{3}}(2\sigma_3 + 2\sigma_6 - \sigma_1 - \sigma_2 - \sigma_4 - \sigma_5) \end{aligned}\right\} \quad e_g$$

The π-System. To determine the reducible representation for π−systems each *N* was assigned three cartisian coordinates taking σ bond as band axis following the right hand rule. Each operation that changed coordinate system was considered to have character zero, the one that changed the sign of vector was assigned —1. and the other that left the vector unchanged was assigned +1.[5] Only *X* and *Y* vectors were considered for π bonds. The following reducible representation was obtained.

	E	$8C_3$	$6C_2$	$6C_4$	$3C_2$	i	$6S_4$	$8S_6$	$3\sigma h$	$6\sigma d$
$\Gamma\pi =$	12	0	0	0	−4	0	0	0	0	0

This representation was broken down to the following irreducible representation using equation 1:

$$\Gamma\pi = t_{1g} + t_{2g} + t_{1u} + t_{2u}$$

Referring again to *Oh* character table it was found that Ag^{2+} ion has no t_{1g} and t_{2u} orbitals, while t_{1u} (p_x, p_y, p_z) orbitals are engaged in σ −obonding which leaves only t_{2g} orbitals available for π − bonding with the central metal ion.[6]

The σ and π− orbitals available on the ligand are summarized in Table 1.

On the basis of the preceding discussion an approximate MO schematic was constructed as shown in Fig. 2. This schematic resembles Cu(II) MO as expected. A tetragonal distortion is predicted but since the ligand, tripyridyl, has an extended π — system and after Coordination would be expected to have small flexibility, an averaging effect of distortion can be expected which may be responsible for the apparent equivalence of the six metal–ligand bonds. The system requires detailed analysis of spectral transitions, calculations of group overlap integrals (Gij) and ligand –ligand interaction. This data would give a definite conclusion regarding this system. However, the equivalence of all nitrogens shown by IR spectra is a strong evidence in support of the conclusion drawn above.

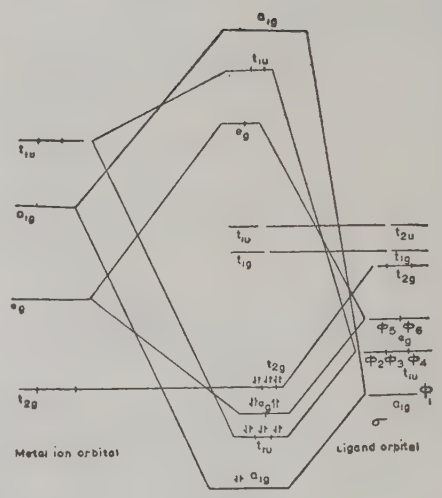

Fig. 2

TABLE 1

Oh	Ligand Group Orbitals
a_{2g}	$\frac{1}{\sqrt{6}}(\sigma_1 + \sigma_2 + \sigma_3 + \sigma_4 + \sigma_5 + \sigma_6)$
e_g	$\frac{1}{2}(\sigma_1 - \sigma_2 + \sigma_4 - \sigma_5)$
	$\frac{1}{2\sqrt{3}}(2\sigma_3 + 2\sigma_6 + \sigma_1 - \sigma_2 - \sigma_4 - \sigma_5)$
t_{1g}	$\frac{1}{2}(\pi_{y1} - \pi_{x3} + \pi_{x4} - \pi_{y6})$
	$\frac{1}{2}(\pi_{x2} - \pi_{y3} + \pi_{y5} - \pi_{y6})$
	$\frac{1}{2}(\pi_{x1} - \pi_{y2} + \pi_{y4} - \pi_{x5})$
t_{1u}	$\frac{1}{\sqrt{2}}(\sigma_1 - \sigma_4)$
	$\frac{1}{2}(\pi_{y2} + \pi_{x3} - \pi_{x5} - \pi_{y6})$
	$\frac{1}{\sqrt{2}}(\sigma_2 - \sigma_5)$
	$\frac{1}{2}(\pi_{x1} + \pi_{y3} - \pi_{y4} - \pi_{x6})$
	$\frac{1}{\sqrt{2}}(\sigma_3 - \sigma_6)$
	$\frac{1}{2}(\pi_{y1} + \pi_{x2} - \pi_{x4} - \pi_{y5})$
t_{2g}	$\frac{1}{2}(\pi_{y1} + \pi_{x3} + \pi_{x4} + \pi_{y6})$
	$\frac{1}{2}(\pi_{x2} + \pi_{y3} + \pi_{y5} + \pi_{x6})$
	$\frac{1}{2}(\pi_{x1} + \pi_{y2} + \pi_{y4} + \pi_{x5})$
t_{2u}	$\frac{1}{2}(\pi_{y2} - \pi_{x3} - \pi_{x5} + \pi_{x6})$
	$\frac{1}{2}(\pi_{x1} - \pi_{y3} - \pi_{y4} + \pi_{x6})$
	$\frac{1}{2}(\pi_{y1} - \pi_{x2} - \pi_{x4} + \pi_{y5})$

References

1. A. A. Usmani, Ph. D. thesis, City University, London, (1972).
2. H.H. Jaffe, and Milton Orchin, *Symmetry in Chemistry* (J. Willley, New York, 1965), p. 28.
3. C.J. Ballahusen, and H.B. Grey, *Molecular Orbital Theory*, (Benjamin, Nenw York, 1965), p. 92.
4. H.B. Gray, and N.A. Beach, J. Am. Chem. Soc., **85**, 2292 1963.
5. J.N. Murral, S.F.A. Kettle and J.M. Tedder, *Valence Theory* (J. Willey, London, 1965), p. 108.
6. F.A., Cotton, *Chemical Applications of Group Theory* (Intersicience, New York, 1965), p. 222.

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STUDIES ON THE FORMAL REDOX POTENTIAL VALUES OF Co^{3+}/Co^{2+} REGARDING THE VOLUMETRIC USE OF HEXAMMINECOBALT(III) TRICARBONATOCOBALTATE(III)

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Abstract. The values of the measured formal redox potential of Co^{3+}/Co^{2+} system in sulphuric, hydrochloric, perchloric and acetic acids, with regard to the possible use of $Co(NH_6)_3Co(CO_3)_3$ as a volumetric oxidimetric titrant, have been reported.

Hexamminecobalt(III) tricarbonatocobaltate(III) $Co(NH_6)_3.Co(CO_3)_3$, prepared according to the method of Bricker *et al.*,[1] has been successfully used by us for the estimation of a number of organic and inorganic substances.[2–5] Since we have found the titrant very useful for volumetric estimations based on redox measurements, it seemed quite desirable to study its formal redox potentials in the acid media. The values of the measured formal redox potentials of the system in acid media like hydrochloric, sulphuric, acetic and perchloric acids, have been reported here.

Experimental

Reagents. A stuitable solution of $Co(NH_6)_3$-$Co(CO_3)_3$ was prepared and its factor checked after the method described earlier.[1]

The solution of Co(II) which was equimolar with respect to $Co(CO_3)_3$ as given in the above formula, was prepared from analytical grade substance by Merck and its factor was checked according to conventional method.

All other reagents used were either of A.R. grade or equivalent purity.

Apparatus. A pH meter (model 290, Pye, Cambridge) with saturated calomel as reference and platinum foil as indicator electrodes was used for potential measurements.

All officially calibrated glassware (A grade) was used during these investigations.

Procedure. To a 250-ml beaker, exactly equal volumes of Co(III) and Co(II) equimolar solutions were added. To the same beaker water and a particular acid were added so as to give a definite total volume which was of the required exact strength with reference to the particular acid. The contents were mixed with magnetic stirrer and the potential reading was noted on its stabilisation. The procedure was repeated with various acids and their various strengths, previously [1–4] found most suited to be used as media for oxidations of many organic and inorganic substances.

Results and Discussions

Formal Redox Potential of Co^{3+} / Co^{2+} System in Sulphuric Acid. The values of the formal redox potential of Co^{3+}/Co^{2+} system were measured in a total volume of 50 ml which was from 0.03 to 14.83N with regard to sulphuric acid. The values of the formal redox potential increase with increasing concentrations of sulphuric acid but after a certain increase in the concentration of the acid, further increase results in decrease of formal redox potential. The possible explanation could be the complex formation of the Co^{3+} and Co^{2+} with the medium due to higher concentration of acid and low concentration of water. The rise in the measured value shows that from 0.03 to 9.93N acid the increase is about 300 mV, while from 4.96 to 9.93N acid the increase is not so appreciable. This shows that it would not be so advisable to use higher concentrations of sulphuric acid in many of the cases for redox studies with the titrant. Anyhow there is no bar, wherever these higher concentrations are necessarily needed for their use as medium. Sulphuric acid stands second to hydrochloric acid in using it as a medium. On the whole sulphuric acid is a good medium for the study of the oxidizing behaviour of $Co(NH_6)_3Co(CO_3)_3$ (Table 1), and the fact is supported by our previous studies.[2–5]

Formal Redox Potential of Co^{3+} / Co^{2+} System in Hydrochloric Acid. The value of formal redox potential of Co^{3+} / Co^{2+} couple in various strengths of hydrochloric acid measured in a total volume of 50 ml shows a more rapid increase (with increase in acid concentration) than those in sulphuric acid. The rise in the value, with the rise of hydrochloric acid concentration from 0.03 to 0.15N is about 532 mV and with further increase in the acid concentration, although the value of formal redox potential decreases yet up to 4.58N hydrochloric acid the decrease is not more than about 103 mV. This means that this value is fairly constant over a fair range of the acid concentration (0.03–4.58N). Further increase in the acid concentration (5.53N) lowers the value of the formal redox potential comparatively at a higher rate than the increasing rate of the acid. The decrease in the measured values with the increase in the acid concentration could be attributed to the liberation of chlorine, at very high rate, from hydrochloric acid at higher concentration and thereby affecting the potential of the indicating electrode.

Comparing with the values of the measured formal redox potential of Co^{3+} / Co^{2+} system in suplphuric acid, these values in hydrochloric acid media are relatively on higher side. Hence it could be safely concluded, and as observed earlier, [2–4] that hydro-

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Table 1. Formal Redox Potential of the System Co^{3+} / Co^{2+}.

Acid concn (N)	Formal potential vs. S. H. E. (mV*)
H_2SO_4	
0.03	887.00
0.17	1022.00
0.88	1128.50
2.90	1129.50
4.96	1148.50
9.93	1184.75
14.83	930.00
HCl	
0.03	696.00
0.15	1228.50
0.42	1221.00
0.92	1189.50
4.58	1123.50
5.53	998.25
$HClO_4$	
0.04	648.25
0.22	862.00
0.42	915.75
0.88	940.75
1.87	945.75
CH_3COOH	
0.38	458.25
0.97	495.74
2.83	542.00
4.96	578.28
7.63	634.50
11.47	643.25
H_3PO_4	
0.05	400.75
0.43	899.50
0.93	1105.75
4.36	633.66
9.43	568.25
14.00	557.00

*Average of multiple readings.

chloric acid is of more use to the studies of $Co(NH_6)_3$-$Co(CO_3)_3$ for various redox estimations. This acid is suitable for use as medium for showing high formal redox potential values of Co^{3+}/Co^{2+} system when compared with other acids.

Formal Redox Potential of Co^{3+}/Co^{2+} System in Perchloric Acid. The values of the formal redox potential of Co^{3+}–Co^{+2} system in perchloric acid-have an increasing trend with the increase in concentration of the acid (Table 1). After 0.88N acid, further increase does not give appreciable increase in the formal redox potential values. Even then the values exhibited in the media show that this acid stands almost after sulphuric acid. The studies were made in 50 ml as total value of the reactants whereas in case of 0.04N it was 100 ml.

Formal Redox Potential of Co^{3+} / Co^{2+} System in Acetic Acid. The values (Table 1) of the system Co^{3+} / Co^{2+} in various concentrations of acetic acid observe (total volume 50 ml) almost a uniform increase with the increase in the concentration of the acid. The values of the measured formal redox potential show that the acid has limited applications for the studies, whereas it too could be quite important in the estimation of organic compounds which could only be dissolved in it (Table 1.)

Formal Redox Potential of Co^{3+} / Co^{2+} System in Phosphoric Acid. The formal redox potential values in phosphoric acid increase with increasing acid concentrations. The total volume of the reactants was 50 ml with the exception of 0.05N medium where it was 100 ml. The increase in the formal redox potential is about 705 mV with an increase from 0.05 to 0.93N of the acid (Table 1). With further increase in the acid concentration the value falls suddenly and remains almost constant with further higher acid concentrations. This acid like other acids could also be quite safely used for the oxidation studies of $Co(NH_3)_6Co(CO_3)_3$.

References

1. J.A. Baur and C.E. Bricker, Anal. Chem., **37,** 1461 (1965).
2. M. Hanif, M. Saleem and Z. Sheikh, Mikrochim. Acta (submitted).
3. M. Hanif, M. Saleem, and Z. Sheikh, Pakistan J. Sci. Ind, Res., **17,** 91 (1974).
4. M. Hanif, J.I. Toor and S.H. Qureshi, Pakistan J. Sci. Ind. Res., **17,** 14 (1974).
5. M. Hanif and M. Saleem, Pakistan J. Sci. Ind. Res., (accepted).

Short Communication

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DETERMINATION OF PEROXYDISULPHATE BY EDTA

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The standard procedures for the determination of alkali peroxydisulphates, based on redox reactions, are (a) iodometric method[1]. (b) reduction of acidified peroxydisulphate with an excess of ferrous salt, [2,3] followed by the determination of unreacted Fe^{++} by $KMnO_4$ or ceric sulphate,[2] (c) reduction of peroxydisulphate as in (b) followed by the determination of Fe^{+++} by mercurous nitrate,[2] and (d) reduction of peroxydisulphate with excess of oxalic acid[2] followed by the determination of unreacted oxalic acid by $KMnO_4$. Besides these redox reactions, peroxydisulphates dissolve many metals,[4] e.g. zinc (redox potential –0. 7628 V [5,6] disolves in $K_2S_2O_8$ (redox potential 2.01 V [5,7,8] according to the following reaction.[4]

$$Zn + K_2S_2O_8 \longrightarrow Zn\,SO_4 + K_2SO_4$$

The reaction between zinc metal dust and $K_2S_2O_8$ is exothermic and does not appear to be accompanied by the evolution of any gas. The solution, obtained after shaking aqueous $K_2S_2O_8$ with zinc metal dust for 5 min, did not liberate iodine from KI and gave positive test for Zn^+. According to these observations the reaction between zinc metal dust and $K_2S_2O_8$ appeared to be quantitative and reasonably fast. In the present communication, a method is described for the determination of potassium peroxydisulphate by reacting it with an excess of zinc metal dust followed by the determination of Zn^{++} by EDTA.[2]

Experimental

Reagents. (1) Ethylenediaminetetraacetic acid disodium salt (EDTA) AR. (2) Erio-chrome Black T indicator[2] (0.2 g of the dyestuff dissolved in 15 ml of triethanol amine and 5 ml of absolute alcohol). (3) Zinc metal dust AR. (4) Potassium peroxydisulphate AR. (5) Buffer solution (142 ml of conc NH_4OH and 17.5 g of NH_4Cl AR).

Method. A standard $K_2S_2O_8$ solution in a dry conical flask, was shaken with zinc metal dust, about 1 g more than the required quantity, for 5–10 min. The solution was filtered through a dry filter paper (Whatman No. 42) into a dry flask. A 25 ml aliquot of the filtrate was diluted to 100 ml, followed by the addition of 2 ml of the buffer solution and a few drops of the indicator. The solution was titrated with standard EDTA solution until the colour changed from wine red to blue. Potassium peroxydisulphate is calculated as given below.

1.0 ml 0.1M EDTA ≡ 6.538 mg Zn ≡ 27.04 mg $K_2S_2O_8$

This procedure was checked against ferrous ammonium sulphate–permanganante method.[3] The result of these analyses are given in Table 1. The time dependence of the reaction between zinc metal dust and $K_2S_2O_8$ is shown in Table 2.

Results and Discussions

The accuracy of the Zn–EDTA method, for the determination of $K_2S_2O_8$, is comparable with the ferrous ammonium sulphate method[2] (Table 1). In both the procedures the error in the assayed values of $K_2S_2O_8$ is not more than ± 0.5%. Determination of peroxydisulphates by the methods (a–d) is cumbersome because freshly prepared reagents are required and they need standardization against primary standards. However, in the Zn – EDTA method, no such difficulty is encountered; standard solution of EDTA can be stored for long periods without any appreciable deterioration. Moreover, complete reduction of potassium peroxydisulphate. by Fe^{++} takes 10–15 min[3,2] while the reaction between zinc metal dust and $K_2S_2O_8$ is completed in about 5 min. In place of zinc, magnesium metal powder also be used, perhaps more effectively for dilute and weakly alkaline solutions' of peroxydisulphates because the redox potential of zinc is–0.7628 [5,6] while that of magnesium is–2.37 V,[5] the reaction between magnesium metal powder and $K_2S_2O_8$ is likely to be very fast.

TABLE 1. DETERMINATION OF $K_2S_2O_8$ BY Zn–EDTA METHOD.

Wt of $D_2S_2O_8$ (g/25ml)	Wt. of $K_2S_2O_8$ found (g/25 ml)	
	Zn–EDTA method	Fe^{++} method[2]
0·5069	0·5082	0·5082
0·5089	0·5055	0·5062
0·5069	0·5062	0·5062
0·5069	0·5082	0·5055
0·5089	0·5062	0·5082
0·6764	0·6758	0·6782
0·6764	0·6744	0·6772
0·6764	0·6772	0·5658
0·6764	0·6758	0·6782
0·6764	0·6758	0·6772

TABLE 2. TIME DEPENDENCE (IN MIN) OF THE REACTION BETWEEN ZINC METAL DUST AND $K_2S_2O_8$.

Wt. of $K_2S_2O_8$ (g/25 ml)	Wt. of $K_2S_2O_8$ found (g/25 ml)			
	2 min	3 min	5 min	10 min
0·3380	0·2677	0·2123	0·3375	0·3380
0·3380	0·2681	0·3232	0·3380	0·3387
0·3380	0·2704	0·3232	0·3366	0·3387
0·5063	0·4056	0·4780	0·5080	0·5073
0·5063	0·4022	0·4860	0·5068	0·5058
0·5063	0·4070	0·4860	0·5068	0·5073

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References

1. I.M. Kolthoff and E.M. Carr, Anal. Chem., **25,** 296 (1953).
2. A.I. Vogel, *A Textbook of Quantitative Inorganic Analysis* (Longmans Green, London, 1961), third edition.
3. G. Charlot and Denise Bezier, *Quantitative Inorganic Analysis*, English translation (Methuen, London, 1957).
4. J.R. Partington, *General and Inorganic Chemistry* (Macmillan, London, 1967), fourth edition.
5. W. R. Kneen, M.J.W. Rogers and P. Simpson, *Chemistry Facts, Patterns and Principles*, Table A 14, Appendices. (Addison-Wesley, London, 1972).
6. *Langes Handbook of Chemistry* (McGraw-Hill New York, 1967), tenth edition.
7. F.A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry* (Interscience, London, 1972) third edition.
8. R.C. Brasted *Comprehensive Inorganic Chemistry* (D. Van Nostrand, New York, 1961), vol. viii.

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GENERA OF SUBFAMILY LINDENIINAE (GOMPHIDAE ANISOPTERA : ODONATA) WITH DESCRIPTIONS OF TWO NEW SPECIES FROM PAKISTAN

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Abstract. The distribution and amended characters of the genera *Ictinogomphus* Cowley and *Gomphidia* Selys are included in this paper. Detailed description of two new species, viz. *Ictinogomphus alaquopterus* and *Ictinogomphus pugnovittatus* and modified characters and new record of distribution of *Gomphidia t-nigrum* Selys are given.

Genus *Ictinogomphus* Cowley

This genus is found in the eastern region of southern Asia, Australia, and the tropical and subtropical Ethiopia. Specimens of two species of this genus have been collected from Pakistan, both of which are new. These specimens agree with Fraser's description of 1934 of this genus except the following characters: primary antenodal nervures 1st and any of the 6th to 8th; arc situated between 1st and 2nd antenodal nervures, or rarely opposite the 2nd; 6–8 transverse nervures between sectors of arc from arc to the point of bifurcation of Rs in hind wing; a distinct anal loop made up of 4–5 cells present or not defined; anal triangle 5–7 celled; subtrigone in fore wing 2 (rarely 3) celled.

Ictinogomphus alaquopterus sp. nov.
(Figs. 1–5)

Male

Head. Labium greenish-yellow with its anterior margin brownish; labrum, clypeus and frons yellowish-green, the former broadly black on its anterior border, very narrowly on lateral borders, and also with a blackish-brown, narrow basal line; postclypeus with a minute, slightly elongated, black spot on each side of its middle; frons with a transverse black stripe at the lower part of the front rising up rather broadly from the middle almost to the crest to separate two lateral areas and with a broad triangular black basal spot; vertex and vesicle black; occiput greenish-yellow, narrowly bordered with brownish-black and with a fringe of small yellow hair on its hind border; eyes olivaceous.

Prothorax. Black; posterior lobe with a transverse yellow stripe in the middle.

Pterothorax. Black, marked with yellowish-green as follows: mesothoracic collar completely, 2 short and narrower oblique dorsal stripes with their pointed anterior-ends separated from the mesothoracic collar; a humeral stripe represented by an upper minute triangular spot and a lower narrow stripe covering lower half of humeral suture and slightly broadening on lower end; The tergal spots; sides, except two broad black bands, one at the level of anterolateral suture which is narrowed below spiracle, another on posterolateral suture; spiracle cuts the anterior border of the broad complete mid-lateral yellowish-green stripe, another large yellow spot separated just below the lower end of this stripe; posterior border of metepimeron black separating a small triangular yellow area; metepimeron obliquely furrowed just below hind-wing bases, this furrow with a minute black spot in its middle.

Legs. Black; coxae and trochanters spotted with yellow; hind femur with a short and very fine basal yellow streak on posterior surface and with two rows of spines, those of the outer row closely-set and small near the base, followed by 6 or 7 long and robust gradually shortening towards the distal end.

Wings. Hyaline blackish-brown, limited to extreme bases in inferior costal and cubital spaces in all wings; costa olivaceous up to pterostigma; nodal index $\frac{12-23}{12-16}$ $\frac{22-13}{16-11}$; bridge 7–8 celled; trigone 3–4 celled in fore wing, always 3-celled in hind wing; anal triangle normally 5-celled (very rarely 6); cubital space traversed by 3 nervures (excluding the base of subtrigone) in fore wing, 2 in hind wing; pterostigma black covering 5–6 cells; membrane blackish-brown at base otherwise whitish.

Abdomen. Black, marked with yellow as follows: segment 1 with a broad, dorsal stripe not reaching anterior base, this stripe extends narrowly along apical borders of segment on sides, and ends in a minute rounded black spot near ventral border of segment and continues back with a broad lateral spot on segment 2 which covers but does not extend beyond oreillet; segment 2 with a large triangular dorsal basal marking extending back as a short tongue-like prolongation not reaching apical border; segment 3 yellow in basal half; segments 4–6 marked with yellow-like segment 3 but this marking not reaching down on sides up to ventral borders of segments, and traversed by a short black prolongation at mid-dorsal carina;

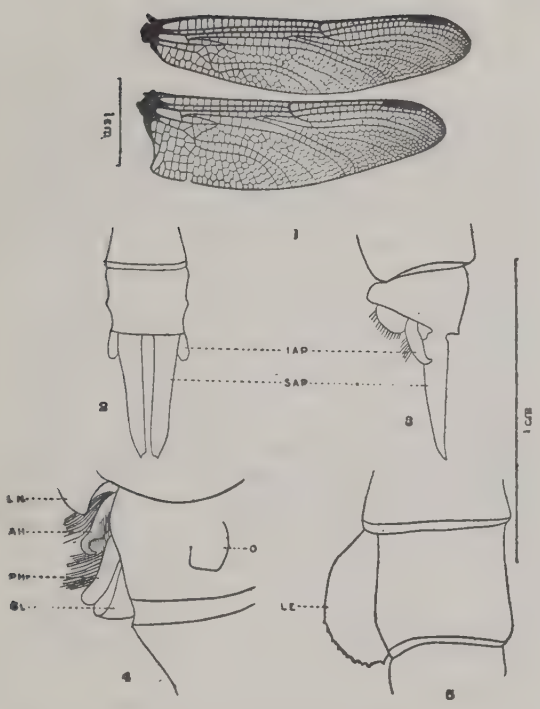

Fig. 1. Fore and hind wings of male *Ictinogomphus ala quopterus* sp. nov.

Fig. 2. Superior and inferior anal appendages of male *Ictinogomphus alaquopterus* sp. nov. Dorsal view.

Fig. 3. Same. lateral view.

Fig. 4. Secondary genitalia of male *Ictinogomphus alaquopterus* sp. nov. Lateral view.

Fig. 5. Lateral expansion of 8th abdominal segment of male *Ictinogomphus alaquopterus* sp. nov. showing spines. Lateral view.

segments 7 and 8 with more than basal halves yellow, with black indenting the yellow on dorsum; large, ventral semicircular expansions of segment 8 black; segment 9 with two broad, basal lateral spots, broadly separated on dorsum by black and two small apical lateral spots, one on each side; segment 10 with a bow-like stripe of yellow on the dorsum and sides; anal appendages black; superiors very long, almost as long as the last two segments taken together, cylindrical, slightly narrowed at apices, each with an outer minute spine; inferior nearly one-third the length of superiors, very deeply and broadly bifid into diverging processes (if seen in profile).

Genitalia. Lamina very large and tumid, having a basal row of 5 minute spines on either end, its free border greatly depressed and sunk, shallowly and very broadly notched from the middle (if seen from below); anterior hamule robust, strongly hooked; posterior hamule rather long, robust, tongue-like process (if seen in profile); anterior hamule not clearly visible and hidden beneath long, stout yellow hair (lesser than *I. pugnovittatus* sp. nov.) directed inwards and covering the genital orifice.

Female. Not known.

Measurements of Male. Abdomen 50 mm. Hind wing 39 mm. Pterostigma 5 mm. Superior anal appendages 4 mm.

Holotype. One male collected from Rawal lake (Rawalpindi) on August 11, 1966 and deposited in the Entomological Museum, University of Agriculture, Lyallpur.

This species closely resembles *I. rapax* from which it can be separated very easily by the following characters: anal loop in hind wings not defined; a third complete mediolateral broad yellow stripe is present on the mediolateral broad black band instead of 1–3 yellow spots on pterothorax in *I. rapax*; labrum without a median black basal prolongation; segment 7 yellow with a broad apical black ring.

Ictinogomphus pugnovittatus sp. nov.
(Figs. 6–10)

Male

Head. Labium pale greenish-yellow, with the anterior margin brown; mandibles red with greenish-yellow bases and reddish-brown inner borders; labrum, clypeus and frons pale greenish-yellow, the former with its anterior border very broadly black, its base with a very narrow brownish stripe; postclypeus with two minute submedial obsolete brownish spots; a narrow blackish-brown stripe on transverse sulcus between frons and clypeus; frons with a broad, basal, triangular brownish-black marking; vertex and vesicle entirely black; occiput greenish-yellow, narrowly bordered with blackish-brown, its hind border with a fringe of short brown hair; back of eyes black, yellowish-brown above, blackish-brown on sides.

Prothorax. Anterior and middle lobes blackish-brown, the latter with lateral ends broadly yellow; posterior lobe yellow with its hind border broadly brownish-black.

Pterothorax. Brownish-black, marked with greenish-yellow as follows: A complete mesothoracic collar, a broad dorsal oblique stripe on either side with its base on the antealar sinus, pointed below and very slightly separated from the mesothoracic collar; broad humeral stripe, slightly penetrated by the brownish-black band on humeral suture near its upper end which is knobbed near antealar sinus, the latter with a large elongated central spot, giving back two short lateral arms from its posterior end, and sending a very fine line to mid-dorsal thoracic carina from its anterior end; the tergum spotted with yellow; sides greenish-yellow, with brownish-black broader stripes, one on anterolateral suture, and the other on posterolateral suture; this latter gives out an anterior, curved, very narrow line below the spiracle from near its lower end which separates below a broad yellow area.

Legs. Black; coxae and trochanters greenish yellow; outer and posterior surfaces of all femora reddish-brown and this colour not reaching quite up to distal ends; inner surface of anterior femora broadly yellowish-green; a fine reddish-brown obs-

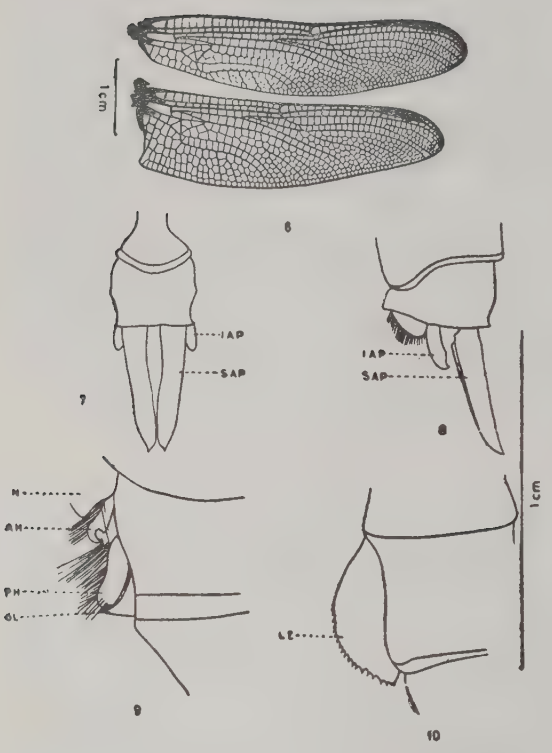

Fig. 6. Fore and hind wings of male *Ictinogomphus pugnovittatus* sp. nov.

Fig. 7. Superior and inferior anal appendages of male *Ictinogomphus pugnovittatus* sp. nov. Dorsal view.

Fig. 8. Same. Lateral view.

Fig. 9. Secondary genitalia of male *Ictinogomphus pugnovittatus* sp. nov. Lateral view.

Fig. 10. Lateral expansion of 8th abdominal segment of male *Ictinogomphus pugnovittatus* sp. nov. showing spines. Lateral view.

AH, anterior hamule; GL, genital lobe; IAP, inferior anal appendage; LE, lateral expansion; LN, lamina; O, oreillet; PH, posterior hamule; and SAP superior anal appendage.

cure line on the outer sides of tibiae; hind femur with an outer and inner row of short, stout, closely-set spines at base, then 4–5 very long and robust widely-spaced spines, again shorter ones near distal end.

Wings. Hyaline, a yellowish-brown basal marking extending as far as 1st antenodal nervure, 1st cubital nervure in all wings, and in addition 1–2 basal postanal cells in fore wing and 2–3 of the anal triangle along the membrane in hind wing; extreme apices frosted with milk-white colour; costa olivaceous up to pterostigma; anal triangle with 6–7 cells in 2 rows; arc very slightly proximal to 2nd antenodal nervure or opposite to it; hypertrigones and trigones 3-celled; subtrigone in fore wing 2–3 celled, in hind wing entire; cubital nervures 3 in fore wing, 2 in hind wing; nodal index $\frac{12-20}{13-15}$ $\frac{23-12}{17-13}$; bridge 7–8 celled; pterostigma black reddish-brown, covering 5–6 cells; membrane with basal half blackish-brown, apical half white.

Abdomen. Compressed, brownish-black, marked with yellow as follows: segments 1 and 2 with sides very broadly yellowish, the former with basal half on dorsum, the latter with a large triangular marking on the dorsum not reaching back to apical border; segments 3–6 with basal halves and this colour not extending to ventral borders on sides except on segment 3; segment 7 yellow with a diffused basal and a very narrow apical ring, these rings giving some black colouration on sides; segment 8 yellow with a fine apical stripe of blackish-brown; semicircular expansions of ventral borders black with well-developed stout denticles along borders; segment 9 blackish-brown with lateral narrow basal and apical yellow spots; segment 10 entirely yellow. Anal appendages: Superiors yellowish-white, their apices and bases slightly brownish, very long and stout, double the length of segment 10, subcylindrical, with a narrow space in between and almost meeting at apices, the latter slightly narrowed having an outer minute spine; inferior black, about one-third the length of superiors, deeply and broadly bifid into diverging triangular processes (if seen from below), apices are pointed and turned upwards (if seen in profile).

Genitalia. Lamina very large and tumid, having a basal row of 3 minute spines on either end, its free border greatly depressed and sunk, and broadly and deeply notched from the middle (if seen from below); anterior hamule short robust, strongly hooked; posterior hamule short and broad tongue-like process (if seen in profile); all the parts not clearly visible, and hidden beneath long, stout, yellow hair directed inwards and covering the genital orifice; genital lobe short, funnel-shaped.

Female. Not known.

Measurements of Male. Abdomen 51 mm. Hind wing 40 mm. Pterostigma 5.50 mm. Superior anal appendages 3.75 mm.

Holotype. One male collected from Balloki Head (Sheikhupura) on July 23, 1966 and deposited in the Entomological Museum, University of Agriculture, Layllpur.

This species runs very close to *I. angulosus* from which it can be separated very easily by the following characters: labrum yellow with its anterior border and heavily black; vertex black ; an additional broad yellow humeral strips parallel to the broad dorsal oblique stripe is present on each side, this humeral stripe narrows near its upper end and then becomes abruptly knobbed, the knob rests on the broder of the antealar sinus; triangle 6–7 celled; the large leaf-like expansions on segment 8 with numerous stout denticles on free borders; abdominal markings differ very greatly from *I. angulosus*,especially segment 10 which is entirely yellow; anal appendages are yellowish-white and double the length of segment 10.

Genus *Gomphidia* Selys

This genus is found in oriental region to east Asia and tropical continental Africa. Following amendments in Fraser's description of 1934 of this genus are suggested on the basis of characters of male from Pakistan: primary antenodal nervures the 1st and any of 5th–7th; position of the arc variable, between 1st and 2nd or 2nd and 3rd antenodal nervures or opposite the 2nd.

Gomphidia t-nigrum Selys

A single male of this species from Pakistan agrees with Fraser's description of 1934 except the following:

Male. Pterothorax with two minute rounded greenish-yellow spots just outer to bases of oblique dorsal stripes on either side. Legs according to Fraser's description, but posterior side of extreme distal ends of femora marked with yellow and with a narrow streak of yellow colour not reaching distal end; fore femora with flexor surface very broadly greenish-yellow, this colour not reaching distal end. Wings hyaline with a yellow colouration at the extreme bases in cubital spaces; membrane dirty white. Abdominal segments 3–7 with basal half yellow; from posterior half of segment 7–9 with slight black colour diffusely spread, each of these segments with a large distinct lateral black marking on either side along ventral borders; segment 10 yellow on dorsum, this colour extending diffusely on sides; anal appendages brownish black.

Measurements of Male. Abdomen 53 mm. Hind wing 39 mm. Anal appendages, superior 3.50 mm, inferior 1.50 mm. Pterostigma 4.75–5 mm.

Distribution. Lahore 1, May 10, 1964; Rawalpindi 2, June 7, 1963; Peshawar 1, June 2, 1964.

New Record. Peshawar.

Type. From northern India (locality unknown) is in the Selys collection.[1]

Reference

1. F.C. Fraser, *Fauna of British India* (Taylor and Francis, London, 1934), vol. II.

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EFFICACY OF METHYL EUGENOL AS A MALE ATTRACTANT FOR DACUS ZONATUS (SAUNDERS) DIPTERA: TEPHRITIDAE*

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Abstract. *Dacus zonatus* (Saunders) is a serious pest of guava in Pakistan which is responsible for 25–50% loss of fruit. The efficacy of methyl eugenol as an attractant alone and in different combinations with toxicant, protein hydrolysate, and sugar was explored for the males of this species. A solution of 85% methyl eugenol + 5% naled + 10% sugar (v/v/v) was found to be the best combination for attracting and killing the flies for a longer period.

In Pakistan *Dacus zonatus* (Saunders) is an important pest of fruits like guava, mango, papaya and citrus. The fly causes damage to the extent of 25–50%, and in places where no control measures are applied almost all the guava fruit become infested by the fly especially during summer.[1]

Attractiveness of methyl eugenol for the males of Oriental fruitfly, *Dacus dorsalis* Hendel has been reported and the lure was utilized in testing the male annihilation concept of insect control against this species.[2,3] Steiner *et al.*[4,5] used the male annihilation technique successfully in the Mariana islands to eradicate the Oriental fruitfly, *D. dorsalis* through the aerial distribution of $2\frac{1}{4} \times 2\frac{1}{4} \times \frac{3}{8}$ in fibre board blocks soaked in a solution of the male lure, methyl eugenol, plus naled.

In order to evaluate the effectiveness of methyl eugenol as well as its economical use against *Dacus zonatus* it is imperative that its efficacy of attractiveness is known under our conditions. In the present studies, therefore, the efficacy of methyl eugenol as an attractant for the males of *Dacus zonatus* Saunders alone and in combinations with toxicant (naled), protein hydrolysate and sugar was explored.

Materials and Methods

The experiment was conducted in a 50-acre guava garden near Tandojam and the following combinations were tried:

100% methyl eugenol
95% methyl eugenol + 5% naled
90% methyl eugenol + 5% naled + 5 % protein hydrolysate
85% methyl eugenol + 5% naled + 10% protein hydrolysate
90% methyl eugenol + 5% naled + 5 % sugar
85% methyl eugenol + 5% naled + 10% sugar
85% methyl eugenol + 5% naled + 5% protein hydrolysate + 5% sugar
80% methyl eugenol + 5% naled + 5% protein hydrolysate + 10% sugar
75% methyl eugenol + 5% naled + 10% protein hydrolysate + 10% sugar

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Four ml solution from each of the combinations were injected separately in cotton wicks measuring 5.4 cm long and 1.5 cm dia of the individual one gallon plastic trap. The traps were hung on the guava trees at the height of 4–6 ft above the ground and the experiment was replicated twice.

The record on the number of male flies trapped in each trap was maintained. The data was subjected to analysis of variance, and Duncan's multiple range test was used for testing the significance of the differences between the means.

Results and Discussion

The traps having combinations (ii), (iii), (v) and (vi) captured significantly higher number of male flies than the rest of the combinations under trial (Table 1). In combination (ii) mean male fly catch per trap per fortnight was 9624 while in combinations (iii), (v) and (vi), the male fly catch was 7172, 7610, and 8173 respectively; combination (i) recorded the lowest fly count (3339 flies). This is due to the fact that the flies might have consumed all the lure as there was no poison to kill them.

In order to screen out the best treatment from combinations (ii), (iii) (v) and (vi) another experiment was conducted in the same garden. All the above-mentioned combinations including 100% methyl eugenol [combination (i)] were replicated 10

Table 1. Efficacy of Methyl Eugenol as an Attractant for Males of *D. zonatus* when used alone and in Different Combinations with Insecticide (Naled) Sugar and Protein Hydrolysate During Ausust 4–October 20, 1973.

Combinations	Average no. of male flies captured/trap/fortnight						Mean
	1st	2nd	3rd	4th	5th	6th	
(i)	5733	3965	6415	2049	1749	123	3339
(ii)	7891	6932	19695	11435	6302	5485	9634*
(iii)	8250	5967	15263	7532	3829	2188	7172*
(iv)	5923	4895	15704	6356	2813	1379	6178
(v)	7084	6048	15755	8224	4015	4535	7610*
(vi)	6276	8606	12967	15758	3336	2097	8173*
(vii)	6254	6631	15212	6270	2143	762	6212
(viii)	1155	7784	17595	7930	2773	3126	6727
(ix)	8235	6371	15507	3576	1676	4248	6602

*Combinations (ii), (iii), (v) and (vi) are significant ($P \geqslant 0.01$).

TABLE 2. EFFICACY OF METHYL EUGENOL AS AN ATTRACTANT FOR THE MALES OF *D. zonatus* (SAUNDERS) WHEN USED ALONE AND IN DIFFERENT COMBINATIONS WITH INSECTICIDE (NALED) SUGAR AND PROTEIN HYDROLYSATE DURING MARCH 18–JUNE 3, 1974.

Combinations	Average no. of flies captured/trap/fortnight					Mean
	1st	2nd	3rd	4th	5th	
(i)	124	546	454	198	12	226.8e
(ii)	254	1360	2216	1729	215	1154.6c
(iii)	385	1357	2247	1911	257	1231.4b
(v)	173	974	1365	1528	612	931.00d
(vi)	229	1192	2398	3608	1839	1853.2a

Treatment means followed by different alphabets are segnificantly differ from each other at 1% level.

times. The data (Table 2) show that in order of effectiveness the highest average number (1853) of male flies per trap per fortnight was trapped in combination (vi), followed by combinations (iii), (ii) and (v) where the mean fly catch was 1231, 1155 and 931 respectively. Combination (i) again recorded lowest fly count (267 flies). This experiment was again repeated in an identical manner for a 3-months period (July–Spetember, 1974). The results confirmed the higher efficiency of attractiveness of the combination (vi). Based on these results it is concluded that a solution of 85% methyl eugenol + 5% naled + 10% sugar injected in cotton wicks of dry plastic traps could effectively be used to annihilate the male fly population of *D. zonatus*.

Further studies are planned to find out a suitable thixotrope for distributing the poisoned male lure. Several different thixotropes have been reported in the literature, which could be used asan extender, e.g. Myverol (distilled monoglycerides of lard, i.e. monoesters of glycerol),[6] Cab-o-Sil (an amorphous pyrogenic silica)[7] and Thixcin E (nontoxic chemically inert organic powder).[8]

References

1. R.A. Syed, Procedings of the Seminar on Insect Pest Control (A.E.C., Lahore, 1967), p. 182
2. L.F. Steiner, J. Econ. Entomol., **45**, 241 (1952).
3. L.F. Steiner and P.K.S. Lee, *ibid.*, **48**, 311 (1955).
4. L.F. Steiner, W.C. Mitchell, E.J. Harris, T.T. Kozuma and M. S. Fujimoto, *ibid.*, **58**, 961 (1965).
5. L.F. Steiner, W.G. Hart, E.J. Harris, R.T. Cunningham, K. Ohinata and D.C. Kamakahi, *ibid.*, **63**, 131 (1970).
6. W.G. Hart, L.F. Steiner, R.T. Cunningham, S. Nakagawa and G. Farias, *ibid.*, **59**, 1395 (1966).
7. R.T. Cunningham, L.F. Steiner, K. Ohinata and G.J. Farias, *ibid*,. **63**, 106 (1970).
8. K. Ohinata, L.F. Steiner and R.T. Cunningham, *ibid.*, **64**, 1250 (1971).

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HISTOLOGICAL EXPRESSION OF IN VITRO GROWTH OF EXPLANTS FROM PARTHENOCARPIC NONPARTHENOCARPIC SYCONIA OF FICUS CARICA L., CV. KING

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Abstract. *In vivo* growth of both parthenocarpic (first crop) and pollinated (second crop) syconia of *Ficus carica* cv. King proceeded in three stages, two stages of rapid growth (period I and III) separated by a period of slow growth (period II). To obtain information concerning relative sensitivity to growth regulators of syconium tissues of each crop during development, explants were grown in culture media with and without growth regulators. The regulators used were 2,4-dichlophenoxy acetic acid, kinetin and gibberellic acid, each at two concentrations predetermined as suitable. After 4 weeks of *in vitro* growth microtome sections of these were observed for the evidence of types of histological growth. The wound response was formation of periderm, the extent of which was more in 2,4-D treatments. Some differences were observed in explant growth from two types of syconia at the same stage of development. These differences with their probable significance are described.

The King cultivar of fig (*Ficus carica* L.) produces two crops a year. The first-crop fruits are parthenocarpic and are borne on 1-year-old wood. They usually set at the end of April and mature at the end of June. Although fruitlets of parthenocarpic syconia lack embryos and endosperm, a series of supernumarary ovules develop over a period of 9 weeks or longer.[1] The endocarp of individual fruitlets sclerifies and imparts hardness to the so-called seed. Fruits of the second crop develop on current season's growth. They require caprification (fertilization) which occurs late in June and fruits mature at the end of August. Mature fruits of the first crop are generally larger and their form is more generally tapered from tip to the stem-end. The studies made on the shifts in levels of native harmones during the development of two types of syconia showed quantitative and qualitative differences in total 'free' auxins and acidic gibberellins.[2] Thus the differences exist in: (a) the environmental conditions under which the two crops develop; (b) the size and shape and in internal structure of syconia during their development; and (c) in levels of native harmones during their development. These differences suggested that relative sensitivity of the explants from parthenocarpic and nonparthenocarpic fruits to growth regulators in the culture medium might be different so far as their histological behaviour is concerned. This behaviour as reflected in the histological types of *in vitro* growth might give some clue to the *in vivo* growth of two types of syconia.

Materials and Methods

Measurements of Fruit Growth. The basal first-crop fig on previous season's growth and the basal second-crop fig on current-season's growth on each shoot were tagged for the determination of weekly growth increments. Horizontal diameter of 25 tagged fruits of each crop were measured with a vernier caliper.

Procedures for Culturing Fruit Tissue

Collection of Samples. Fig fruits of both crops were collected at 2-week intervals on the same calender dates for two years. Collections of the first year were used for preliminary tests of the suitability of the medium with and without added growth substances. Explants from ripening figs could not be cultured successfully. Samples were held in a refrigerator and were cultured within 1 week after collection.

Preparation of the Tissues. In preparation for culture, the surface of the fruit was sterilized with 0.3% sodium hypochlorite solution for 15 min followed by washing in sterile water. The fruits were then cut in halves, the outer surface pared, and the buds of flowers and the intervening epidermal wall removed aseptically. Small square segments were cut from the region of maximum diameter and each segment was weighed in a sterilized envelop made from weighing paper.

Composition of the Medium. The basic medium contained one-half the concentration of the micro- and macro-elements in Murashige and Skoog's[3] modified formulation of White's medium. Choline chloride (0.5 mg/l) was added to their list of vitamins and instead of 3, 2% sucrose was used. The source of iron was Fe-EDDHA [sodium ferric ethylene di-(*o*-hydroxyphenyl acetate)]. Bacto-agar (0.8%) was added to solidify the medium. The pH of the medium was adjusted to 6.2 before injection of 10 ml aliquots of medium into 15 × 150 mm test-tubes which were then autoclaved (20 min, 15 p.s.i., 120°).

KN (kinetin–6–furly amino purine) and 2,4-D (2,4-dichlorophenoxy acetic acid) were added to the basic medium beforeautoclaving. GA—gibberellin (K salt of gibberellic acid) was added to the test tubes of warm, unsolidified medium by filtering it through a millipore filter. The following concentrations were used: 0.1 μg/ml KN, 0.5 μg/ml 2,4-D and 10 μg/ml GA and also doubled concentrations of each. In addition all possible combinations of the growth regulators at higher concentrations, respectively, were tested. The basic medium without growth substances was used for control explants. Each weighed fruit segment was planted in a test tube containing 10 ml medium with or without growth substances. The tubes were plugged with cotton. Fifteen to twenty replicate cultured were grown for each test.

Preparation of Explant Sections for Histological Studies. After four weeks in culture, determinations of final weights of the explants were made and 2–3 representative explants from each treatment were fixed in Randolph's fixative. The explants were prepared for serial microtome sectioning and sections were stained in haematoxylene-fast green.

Results and Discussion

In vivo *Growth Pattern of Fig Syconia.* Growth curves for the first (parthenocarpic) and second (pollinated) crops based on diameter are presented in Fig. 1. Each point on the curve is the mean of 25 fruits. As the curves show the growth of the syconia of both crops proceeded in three stages, two stages of rapid growth separated by a period of slow growth, which were designated as periods I, II and III by Crane.[5] The first phase of rapid growth (period I) was due to cell division and some cell enlargement in the syconium wall, while the slow growth phase (period II) resulted from cell enlargement alone. The syconium reached its ultimate size by cell enlargement during the third phase (period III). The circles on the curves (Fig. 1) indicate time of sampling for explant culture in the growth media.

Histological Expression of in vitro *Growth of Fig Syconium.* Types of histological development in tissue explants of fig syconia during *in vitro* growth are shown in the photomicrographs (Figs. 2–8). In studies of growth, i.e. cell enlargement and expression of cell division, in explants subjected to different growth regulator treatments the growth patterns were like certain ones described by others[6,7] dealing with various plant materials. As might be expected, because of the impossibility of duplicating natural conditions for fruit growth when explants are grown in test tubes, none of the growth types found in explants duplicated that of syconium tissue *in vivo.* However, as a basis for comparison of cultured syconium segment with uncultured ones (Fig. 2), a brief histological description of the latter is pertinent.

A segment taken midway between apex and base of syconium wall shows similar structure in figs of both crops. The bulk of a syconium is formed of parenchyma, bounded by outer and inner epidermal layers. The latter is continuous with the epidermis of the pedicels which project from the inner surface of the syconium. Vascular strands and laticifers run through

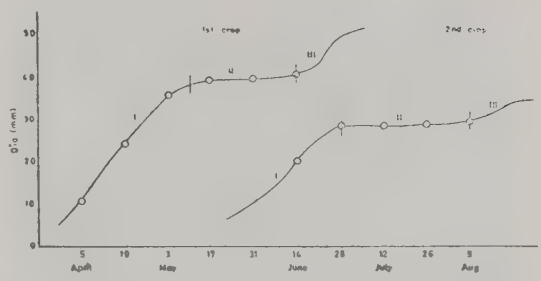

Fig. 1. Growth in diameter of figs of firsts (parthenocarpic) and second (pollinated) crops.

the parenchyma. Adjacent to both outer and inner epidermal layers, a few layers of compactly arranged isodiametric hypodermal cells are present. (Both epidermal and hypodermal layers were removed before explants were cultured). The parenchyma tissue in general is in the form of interlocking chains of cells surrounding air spaces which gradually increase in size as the cells enlarge during development. The largest cells and air spaces are formed midway between inner and outer epidermal layers. The cells surrounding the vascular strands and laticifers are more compactly arranged than cells of parenchyma farther from those structures. The smaller cells and nuclear sizes indicate that probable diploid condition and, therefore, greater possibility of mitotic induction than in the case of presumably polyploid nuclei of the larger parenchyma cells. (Evidences of polysomaty has been found in fig tissue by Bradley and Crane[1]).

Wound Response of Explants. One response to the wounding necessarily inflicted by excision of syconia segments was the formation of a periderm layer a few cells wide beneath the explant surface. Cells of that layer rapidly accumulated much tanning as a rule. Addition of 2,4-D to the medium increased periderm formation. The periderm appeared to greater or lesser extent whether explants were cultured on the basic medium or on media to which growth regulators had been added. In regions of the surface where it did not form, a few peripheral layers of cells deteriorated; they were apparently separated from the inner tissue by changes in the cell walls such as suberisation, which effectively prevented translocation to them as well as dessication of inner tissue.

Types of Growth in Control Explants. In control cultures from first crop figs, little or no mitotic activity took place and the small growth increments resulted entirely from cell enlargement. In controls from second crop figs, however, some cell multiplication occurred, originating primarily in the small cells around the vascular bundles. They were most numerous in explants of the June 14 and July 26 collections. Growth increment in those cultures amounted to more than 100% most of which was attributed to the cell enlargement, however.

Types of Growth in Explants on Culture Media Containing Growth Regulators. Cell Multiplication: Cell proliferation was initiated very close to cut surfaces (Figs. 4, 8 and 9) as well as in deeper regions of the explants (Figs. 3 and 5). In some cases evidence

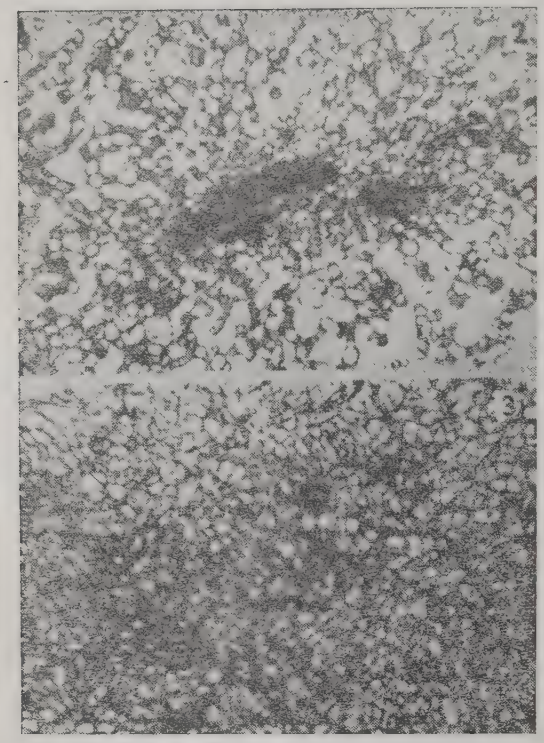

Fig. 2. Typical structure of tissue midway between epidermal layers of a mature syconium showing chains of cells around air spaces, and parts of vascular bundles (× 100)

Fig. 3. Structure of a typical well-developed callus tissue. In the centre of the central large-celled region are groups of vascular cells, but not clearly distinguished. Note evidence of a short cambium layer near upper right. The culture contained KN and 2,4-D. Darker regions are cells filled with tannins and frequently crushed by surrounding tissues × 100).

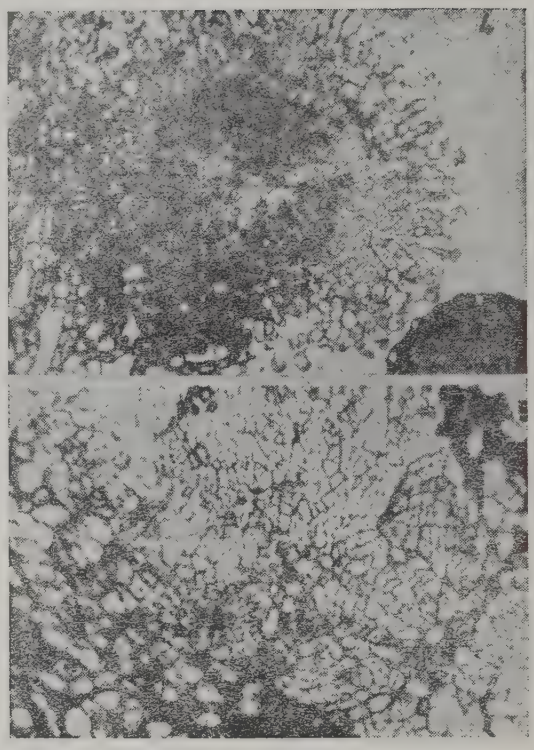

Fig. 4. Structure of a typical callus knob which was still growing at the time of fixation. Early formed cells on the periphery have become enlarged. Dark area shows crushed original syconium tissue which had accumulated tannins, as had also some regions of the callus × 100).

Fig. 5. Part of an extensive internal proliferation surrounded by original crushed explant cells. Internal callus showed similar structure to subsurface callus (Fig. 2), × 100.

of internal proliferation alone was found while in other signs of both subsurface and internal meristematic activity were seen. The proliferations originated from parenchyma around and within vascular strands, particularly in connection with vascular strands lying close and parallel to the explant surface exposed by cutting. The microscopic manifestation of proliferations, when they broke through the overlying cell layers, was the appearance of small callus knobs on explant surface. In some cultures such callus growth appeared when cultures were a week-old or less. Sometimes the callus developed to the extent of crushing adjacent tissue (Fig. 5).

The callus as seen in sections was distinguished from original syconium tissue by the relatively compact arrangement of its cells in contrast to the interlocking chains of parenchyma surrounding sizeable spaces, which is characteristic of the normal syconium tissue (Figs. 2 and 3). A typical highly developed and actively growing callus (Figs. 3–5) displayed considerable heterogeneity in the substructure contained and their determination. Cambial layers of limited span were still giving rise to small cells aligned in rows. The product of pervious cambial activity took the form of differentiated parenchyma of various sizes, still arranged in the linear series that gave evidence of their origin. In older regions of the callus were groups of relatively large parenchyma cells, which had presumably arisen from cambial layers formed early in callus development. Interspersed among these tissues were groups of as many as 12 small cells, oriented in various planes, each group having arisen relatively recently from a single cell as evidenced by the group still being contained within the original expanded cell wall. These groups were comparable to the 'pseudothalli' of Gautheret.[7] In the centre of such callus mass were scattered small groups of short and often distorted vessels and tracheids, sometimes accompanied by clusters of a few equally distorted phloem elements. Vessels and tracheids were reticulately pitted and the vessels had simple perforations. Callus masses having this relatively complex structure developed more extensively near the surface of the explants. At the other extreme of callus types were those in which development apparently had been of short duration and which were composed of somewhat enlarged parenchyma cells usually in serial arrangement, indicating their origin from previously active cambial

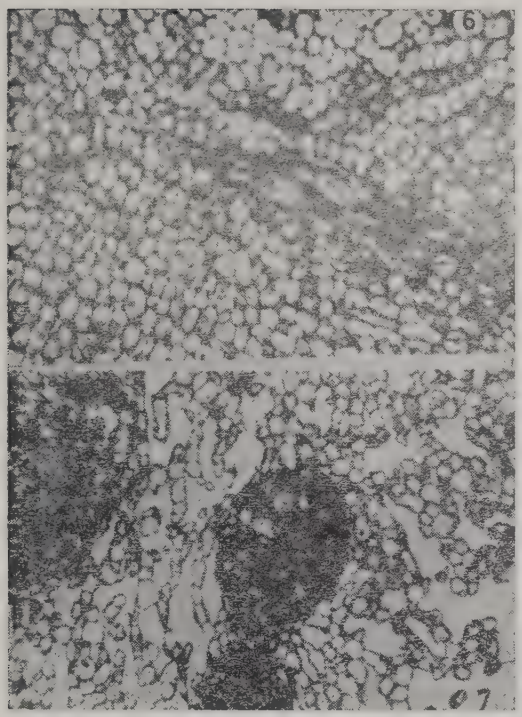

Fig. 6. Section of the explant showing limited internal proliferation, cells of which had differentiated into parenchyma. The proliferation apparently started in cells around the vascular bundle, part of which is shown. GA was added to the medium (× 100).

Fig. 7. Section of explant showing limited internal proliferation, some cells of which had differentiated into vessels and tracheids. KN and GA were added to the medium (× 100).

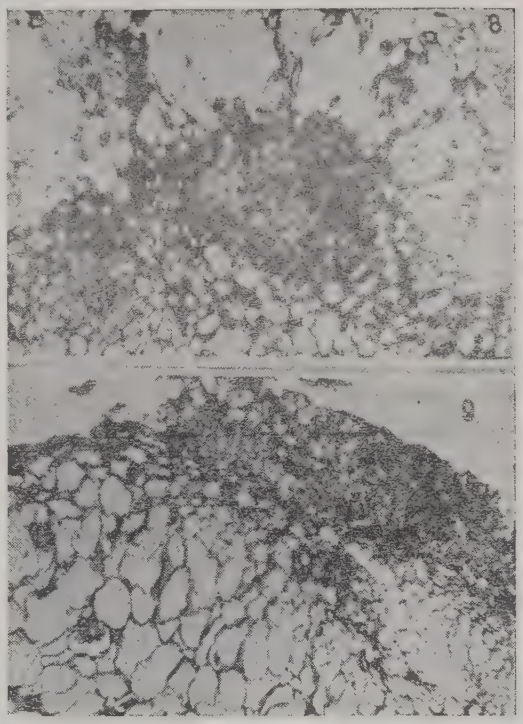

Fig. 8. Section showing a mass of newly formed callus originating from subsurface region of the explant. The extremely large cells near the surface represent the response of original syconium tissue to the KN and 2,4-D added to the culture medium × 100).

Fig. 9. A portion of an explant showing a proliferated mass of tissue from the surface region and greatly enlarged cells in the rest of the explant. KN, 2,4-D and GA were added to the medium (× 100).

layers (Fig. 6). The extent of differentiation and development of callus depended to some extent at least on the growth regulators added to the culture media as is discussed in the later section.

Cell Enlargement. All explants, whether treated with growth regulators or controls, and whether containing cell proliferations or not, included some regions in which cell enlargement had occurred. Explants from both crops at certain developmental stages grew mainly or entirely through cell expansion. Usually cells of the peripheral layers in some regions of the explants enlarged considerably. Addition of certain growth regulators to the media resulted in development of mammoth cells in some regions usually near explant surface (Fig. 8). The response to GA was particularly striking in this respect and to 2,4-D somewhat less so.

Histological Effects of Growth Regulators. The type of histological response to growth regulators, i.e. callus formation in addition to cell enlargement or the latter alone, varied from collection to collection (Table 1). Furthermore, the effect of certain growth regulators on explants from syconia at the same stage of development in some cases differed in two crops. KN induced internal callus growth only in certain samples of each crop. Proliferations were seen in explant sections from samples which responded well to KN alone, indicating interaction of added KN with endogenous auxins. In the first crop they occurred in three collections (April 5, May 31, and June 14), while in the second crop only in the last collection. Growth induced by 2,4-D alone was mainly through cell enlargement, particularly in samples during the period II of the second crop, where it stimulated the most growth. 2,4-D, however, induced relatively small amouts of subsurface as well as internal callus in collections during period II of the first crop (May 17 and 31) and in period I of the second crop (June 14 and 28). Addition of GA alone to the medium, while stimulating growth by cell enlargement in general, also stimulated limited internal cell divisions, the new cells having differentiated into parenchyma only. The cell division occurred in the first two samples of the first crop and in the last sample of each crop.

Both subsurface and inner proliferations were stimulated particularly by additions of both KN and 2,4-D to the medium. In the first crop they were limited to explants from period II but were less

Table 1. Extent of Callus Formation in Explants from Parthenocarpic and Pollinated Fig Syconia Cultured on Media Containing Different Growth Regulators.

Treatment	Parthenocarpic						Pollinated				
	April 5	April 19	May 3	May 18	May 31	June 14	June 14	June 28	July 12	July 26	Aug 9
KN	+				+	+					+
2,4-D				+	+		+	+			
GA	+	+				+					+
KN + 2,4-D				+++	++	+	+++	+++	+++	+++	++
KN + GA							+	+			
2,4-D + GA						+					+
KN + 2,4-D + GA				++	++	+	++	++	++	++	+

+, little; ++, moderate; and +++, Abundant.

frequently seen in the last collection. Explants from syconia of the second crop showed callus formation in all collections treated with this growth regulator combination including those of period I. The addition of GA with KN and 2,4-D decreased the number and size of callus knobs and apparently enhanced cell enlargement (Fig. 9). In explants of two collections of the second crop (June 14 and July 12) where the combination of the 3 regulators stimulated maximum growth, some regions of giant cells were found. The combination of GA and KN stimulated cell enlargement primarily, except in the first 2 collections of the second crop, where the combination induced limited internal proliferations, similar to those caused by GA alone in the first two samples of the first crop. The combination of 2,4-D and GA also stimulated cell enlargement but in the last sample of both crops the combination induced internal proliferation which included differentiation of vascular elements.

References

1. M.V. Bradley and J.C. Crane, Phytomorphology, **15,** 85 (1965).
2. F. Lodhi, M.V. Bradley and J.C. Crane, Plant Physiol., **44,** 555 (1969).
3. T. Murashige and F. Skoog, Physiol. Plant., **15,** 437 (1962).
4. R.R. White, Growth, **7,** 53 (1943).
5. D.C. Crane, Proc. Am. Soc. Hort. Sci., **62,** 237 (1948).
6. C.A. Schoeder, Botan. Gaz., **122,** 198 (1961).
7. R.J. Gautheret, *La Culture des Tissues Vegetaux* (Masson, Paris, France, 1959).

Technology Section

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DETERMINATION OF FAT CONTENT IN GHEE, HYDROGENATED OILS, BUTTER AND POWDERED MILK BY IR SPECTRA

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Abstract. A quick method, using IR spectrophotometry has been investigated for the analysis of (%) fat content in ghee, hydrogenated oils (substituted-ghee), butter and powdered milk. To avoid long procedure of chemical analysis for the determination of fat, this method uses simple preparation of solutions in carbon tetrachloride, under controlled physical conditions, which can be directly used for IR studies. The quantitative estimation has been made by using the 1745 cm^{-1} absorption frequency due to the carbonyl group of glycerides. Correlation of the results obtained by this method as compared with the chemical analysis shows an average variation of $\pm 1\%$ for total fat-content.

In conventional methods[1] for determining the fat-contents of animal and plant materials samples are extracted with suitable solvents and the extracted lipid weighed after removing the solvents. These methods are time-consuming and may not completely extract polar lipids and, therefore, are unsuitable for routine use in quality control laboratories. Refractometric methods[2,3] for determining the fat-content of foodstuffs are also unsuitable because of the interference from nonlipid components and extremely sensitive to fluctuations in temperature.

IR method of analysis offers a means of overcoming these disadvantages. D'yachenko and Samsonov[4] have carried out the analysis of fat by IR using KBr pellet technique. Keisuky and Saito[5] have suggested a thin-film technique using AgCl plate for the analysis of fat in milk which involves a long procedure of sample preparation. The present method uses simple and quick preparation of milk solution in carbon tetrachloride under controlled physical conditions. IR spectra of solution overcome the drawbacks of KBr pellet. Also difficulties may arise for the sample particles to be uniformly distributed in the matrix, which is very important for precise quantitative analysis.

Experimental

Preparation of Standard Fat and Powdered Milk Solutions. Pure butter fat (0.1 g) dried at 100°C was dissolved in 25 ml CCl_4 to provide a standard stock solution A. Two-, four- and eight-fold dilutions of solution A were used as working standards.

Powdered milk (2.8 g, 26% fat) was dissolved in 25 ml CCl_4 containing 2 drops of dil HCl, by warming to 50°C and stirring for 25 min. To remove the moisture, the mixture was filtered into a 25-ml volumetric flask through layer of sodium sulphate and the flask was filled up to the mark with CCl_4. Ten, twenty- and forty-fold dilutions of the above solution were used as working standards, corresponding to 26, 13 and 6.5% fat in powdered milk.

Preparation of Sample Solutions. Sample of ghee (0.1 g), substituted-ghee or butter was dissolved in 25 ml CCl_4 and filtered through a layer of sodium sulphate into a 25-ml volumetric flask after which it was filled up to the mark by CCl_4. The absence of —OH absorption in the region 3635–3610 cm^{-1} confirmed the absence of moisture in the solution.

For the analysis of powdered milk, 2.8 g sample was treated as described for standard powdered milk solution.

Spectra. The spectra were recorded on a Perkin–Elmer 237 spectrophotometer which had a wavelength range from 4000 to 635 cm^{-1} divided into two ranges by different gratings. The ordinate was 0–100% in linear transmittance with an accuracy of $\pm 1\%$ and the reproducibility for identical sampling conditions was within $\pm 1\%$ in the first range used in this work.

Matched cells of 1 mm thickness with NaCl windows were used. Purity of CCl_4 used was checked by IR spectra after redistillation.

Quantitative estimation was made by using baseline measuring technique for absorbance values of the standards and the samples.

Results and Discussion

The standard fat solutions described in the experimental section have been used for drawing a calibration graph against their corresponding ab-

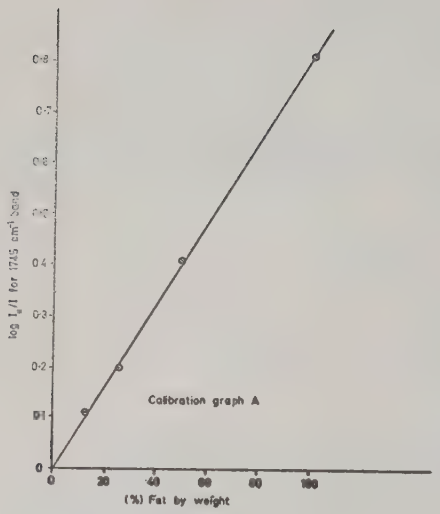

Fig. 1. Absorbance against known percentage of butter fat in CCl_4.

TABLE 1. ANALYSIS OF THE SAMPLES OF GHEE AND BUTTER.

Origin of the sample	log *Io*/*I* for 1745 cm^{-1}	Fat (%) Determined by present method	Fat (%) Determined by chemical method
Ghee (Holland)	0·81	100	100
Hydrogenated oil (Pakistan)	0·66	84	85
Butter (Denmark)	0·63	79	80
Butter (Australia)	0·58	72	72
Butter (Pakistan)	0·63	79	78

sorbance (log *Io*/*I*) for the C—o stretching frequency at 1745 cm^{-1} due to the carbonyl group of glycerides. In order to use this method for routine testing only 0.1 g fat sample is dissolved in CCl_4 and recording the absorbance at 1745 cm^{-1} in 1-mm cell, the fat percentage is noted directly from the calibration graph (Fig. 1).

Table 1 shows the analysis of different type of fat samples by the present method alongwith their analysis by chemical method. The average variation between the two methods is not more than ±1% for total fat content.

Estimation of (%) Fat in Powdered Milk. The determination of fat in powdered milk required suitable conditions for preparation of solution in CCl_4. Several attempts were made and finally the method described in the Experimental section was adopted. The calibration graph B (Fig. 3) was plotted from the concentration against absorbance (log *Io*/*I*) values of 1745 cm^{-1} (Fig. 2 shows the spectra from which absorbance values have been calculated).

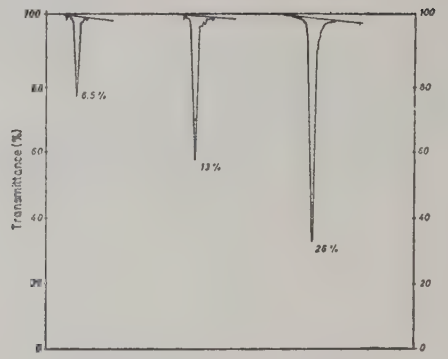

Fig. 2. Transmittance for 1745 cm^{-1} frequency of known percentage of fat in dried milk.

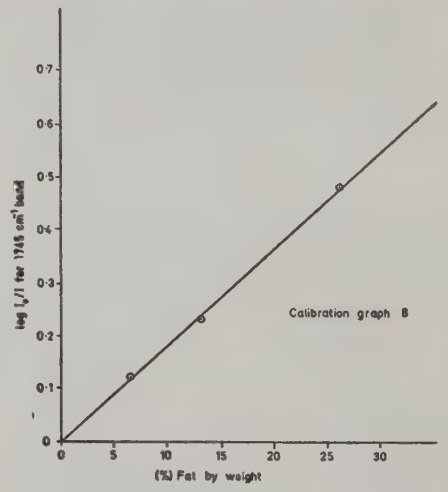

Fig. 3. Absorbance against known percentage of fat of dried milk in CCl_4.

TABLE 2. ANALYSIS OF THE SAMPLES OF POWDERED MILK.

Origin of the samples	log *Io*/*I* for 1745 cm^{-1}	Fat (%) Determined by present method	Fat (%) Quoted on the label
Cow & Gate (Red Cow (England)	0·55	30	28
Cow & Gate (Pakistan)	0·45	25	26
Comila (Belgium)	0·51	27	26
Dano (Denmark)	0·34	18	18
Bebelac (Holland)	0·29	16	18
Arinco, (Denmark)	0·47	26	26
German Export (Germany)	0·51	27	26 (min)
Similac (Holland)	0·45	25	26

Eight samples of different brands of milk have been tested by this method and results are shown in Table 2 alongwith the percentage of fat given on the label of the containers. Three samples have also been tested by chemical method and show a variation of $\pm 1\%$ for total fat, as compared by this method.

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References

1. Society of Public Analysis, Reports of Subcommittee; Analyst, **58,** 203 (1933).
2. A.O.C.S. Official Method, Cc 7-25, Chicago (Corrected 1951).
3. Yuri Lyalikov, *Physico Chemical Analysis* (Mir, Moscow, 1968), p.130.
4. P.D'yachenko and Yu Samsonov, Molochn Prom., **25,** 11 (1964).
5. K. Horitsu and Z. Saito, Hirosaki Daigakn Nogakuka Gakujutsu Hokoku, **12,** 86 (1966).

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STANDARDIZATION OF A PROCESS FOR THE PRODUCTION OF MANGO-FLAVOURED MILK

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Abstract. A standardized process was developed for the production of mango-flavoured milk in Pakistan. Powdered skim milk, fresh mango pulp and canesugar were used as basic ingredients. 16 out of 50 combinations of pectin, gelatin and guar-gum were tested as stabilizers. The effect of room (80–95°F) and refrigerator (40–45°F) storage on their important physicochemical and organoleptic characteristics was studied. Maximum shelf-life was 348 hr at 40–45°F in contrast to 25½ hr at 80–95°F. Gelatin proved best as a stabilizer while the pectin blends were better organoleptically. Lastly, 4 recipes for the preparation of mango-milk blends are given.

Milk is an ideal food and an excellent beverage. In Pakistan where its consumption is 50% less than the recommended international levels, only 23% of the total milk supply is consumed as fluid milk. At the same time Pakistan ranks second in the mango-growing world. The quick perishability of mangoes as well as milk causes heavy losses to the national health and economy.

The present paper, therefore, is an attempt to develop standardized process for the production of a stable mango-milk blend in order to increase the consumption of delicious milk foods fortified by nice fruit flavours, vitamins and valued minerals. Realizing the importance of fruit-flavoured milk, attempts were made to develop technical formulae for the luscious mango-milk blends with better keeping quality without affecting their overall wholesomeness and nutritional value.

Charley[3] utilized various fruit syrups and cordials to produce stable fruit-flavoured milk using sulphur dioxide and sodium benzoate as preservatives. He found that strawberry and raspberry flavours decreased the pH of the blends most rapidly when added to milk. Sattar and Bhatti[14] standardized the manufacture of strawberry-milk shakes in Pakistan. Shih[15] in Nationalist China, produced flavoured-milks using pineapple, essence, sugar and pineapple juice in varying proportions.

Hughes[8] combined orange juice with milk without curdling using gelatin at a special temperature. Bhatti *et al.*[2] worked out a formula for stable orange-beverage base and orange-flavoured milk shake by combining orange-beverage base and fluid milk using citrus-pectin solution as a stabilizer. They found that vitamin C loss in the skim milk blends was greater than in whole-milk blends.

Monzini and Bognetti[11] prepared mixtures of milk and fruit pulps having a pH less than 4.6 using viscous solutions of pectin and carboxy-methyl cellulose as stabilizer. Smith[16] described a patent on stabilized liquid vanilla milk shake mixes using glycol monostearate.

Rembowski *et al.*[12] have tested various stabilizers in the manufacture of milk-fruit mixes. They found that pectin with a high degree of esterification of methoxy groupings proved best as a stabilizer. Hendrick *el al.*[6] studied the possibility of manufacturing cherry-flavoured milk drinks and other dairy products using pectin, carboxymethyl-cellulose and and various buffering salts such as dipotassium hydrogen phosphate. Gorecka *et al.*[5] reported that high temperature of storage leads to the curding of milk case in and to unfavourable colour changes in milk-fruit beverages.

Materials and Methods

Fresh suckling mangoe's pulp obtained manually, defatted milk powder, and cane sugar were utilized as basic ingredients. Milk powder was reconstituted on the basis of 10% solids-not-fat. It was pasteurized at 185–195°F for ½ hr. Strained mango pulp was separately heat-treated at 140–150°F for ½ hr before mixing it with milk.

After cooling, calculated volumes of mango-milk blends were made by adding 5, 10, 15, 20 and 25% of the pulp by volume. Aqueous solutions (2%) of the three stabilizers viz, pectin, gelatin and guar gum were also added to the blends at the rate of 0, 10, 20, and 30% by volume. Finally, the calculated amounts of the cane sugar were added to each blend to fix its initial °Brix at 16.

Out of 50, only 16 blends were selected after a preliminary organoleptic examination and after observing their one day's stability in the refrigerator (Table 1). These blends were then bottled, processed at 143.5–145.5°F for 30 min and finally stored at room (80–95°F) and refrigerator (40–45°F) temperatures (Table 2). The following physicochemical and organoleptic characteristics were evaluated after every 12 hr interval at room storage and after every 24-hr interval at refrigerator storage. The observations were regularly recorded in 2 replications until spoilage occurred.

TABLE 1

Treatment codes	Milk-pulp-stabilizer ratios	Scores obtained out of 50 — Judges A	B	C	D	E	F	Average scores
3 *Pectin-stabilized blends*								
A–1	85: 5:10	27	23	31	32	41	25	29·8
D–1	80:10:10	27	27	38	32	38	34	32·7
G–1	75:15:10	32	21	40	30	34	30	31·2
8-*Gelatin-stabilized blends*								
B–1	85: 5:10	33	10	34	27	21	26	25·2
B–2	75: 5:20	32	10	28	26	20	27	23·8
B–3	60:10:30	25	15	36	29	29	30	27·3
H–1	75:15-10	27	10	36	27	26	27	25·5
H–2	65:15:20	24	10	22	31	26	28	23·5
K–1	70:20:10	28	15	38	33	31	30	29·2
K–2	60:20:20	28	15	35	35	29	30	28·7
K–3	50:20:30	25	10	32	37	32	31	27·8
5-*Nonstabilized blends*								
S–1	95: 5: 0	33	10	25	23	20	22	23·2
S–2	90:10: 0	28	10	25	25	29	26	23·8
S–3	85:15: 0	25	10	25	27	28	24	23·2
S–4	80:20: 0	30	12	27	33	31	32	27·5
S–5	75:25: 0	33	11	26	30	35	28	27·2

TABLE 2. ANALYSIS OF VARIANCE.

Source of variation	D.F.	S.O.	M.S.	F.R.
Judges	5	3379·344	675·869	334·589*
Treatments	15	847·573	56·505	27·973*
Error	75	151·490	2·020	
Total	95	4378·407	46·088	

*Highly significant.

$Cd_1 = (2{\cdot}020 \times 2/6)\,\frac{1}{2} \times 1.99 = 1{\cdot}6328$
$Cd_2 = (2{\cdot}020 \times 2/6)\,\frac{1}{2} \times 2.65 = 2{\cdot}1743$

1. °Brix were read from the Abbe's refractometer.[7]
2. Visual colours were compared with standard colours in a colour dictionary by Maerz and Paul.[10]
3. Total solids (% w/w) were determined by a gravimetric method described by A.P.H.A.[1]
4. pH was read from the pH meter.[13]
5. Acidity (% w/w) as lactic acid was determined by a method given by Horwitz.[7]
6. Vitamin C was assessed by 2, 6-dichlorophenol–indophenol visual titration method as reported by Freed.[4]
7. Organoleptic examination was conducted under the instructions given by Krum.[9]

The panel's scores and the shelf lives of the blends as affected by the 2 temperatures' level were separately examined for significant differences by the analysis of variance technique.

Results and Discussions

The highly significant differences among the combinations' scores at 1% level of significance during the course of preliminary organoleptic examination are given in Table 1. It was striking to note that all those combinations which contained guar gum stabilizer were thoroughly eliminated and could not find a place within the 16 selected blends. While studying the effect of stabilizers on the shelf life of the blends, it was found that all the blends had insignificant differences in their shelf lives at room temperature at 5% level of significance. But at the refrigerator storage, stability of all blends increased in general. The pectin-stabilized blends were the least stable surviving for 48–96 hr nonstabilized blends were in between with an average shelf lives of 72–216 hr while the gelatin-stabilized blends were the best in their stability and their shelf lives ranged from 48 to 348 hr. Stabilizers had little resisting effect on pH and acidity of the blends. Pectin-stabilized blends superseded all blends in their organoleptic quality.

TABLE 3. AVERAGE GRADES IN DESCENDING ORDER.

D–1	G–1	A–1	K–1	K–2	K–3	S–4	E–3	S–5	H–1	B–1	B–2	S–2	H–2
32.7	31.2	29.9	29.2	28.7	27.8	27.5	27.3	27.2	25.5	25.2	23.8	23.8	23.5

S–3	S–1
23.2	22.2

TABLE 4. EFFECT OF TIME AND TEMPERATURE ON THE CHARACTERISTICS OF THE MANGO-FLAVOURED MILK BLENDS AT THE BEGINNING AND END OF THE ROOM TEMPERATURE STORAGE (80-95°F)

Characteristics of the mango-milk blends	Storage	
	Beginning	End
5-Nonstabilized Mango-Milk Blends(Avg. Shelf Life=13.0-18.5 hr)		
°Brix	16·000	16·000
pH	5·600- 6·250	4·900- 5·800
Acidity as lactic acid(%w/v)	0·179- 0·249	0·227- 0·315
Total solids (%w/w)	16·013-16·099	15·977-16·083
Vit C (mg/100 ml)	0·213- 1·029	0·138- 0·550
3-Pectin-Stabilized Mango-Milk Blends (Avg. Shelf Life=13.0-15·0 hr)		
°Brix	16·00	16·00
pH	5·875- 6·075	5·75 - 5·750
Acidity as lactic acid(%w/v)	0·198- 0·222	0·235- 0·260
Total solids (%w/w)	16·057-16·164	16·005-16·108
Vit C (mg/100 ml)	0·179- 0·653	0·133- 0·543
8-Gelatin-Stabilized Mango-Milk Blends (Avg. Shelf-Life=13·5R25·5 hr)		
°Brix	16·000	16·000
pH	5·925- 6·550	5·500- 6·375
Acidity as lactic acid(%w/v)	0·122- 0·215	0·160- 0·270
Total solids (%w/w)	15·948-16·007	16·002-16·300
Vit C(mg/100 ml)	0·173- 0·874	0·105- 0·435

TABLE 5. EFFECT OF TIME AND TEMPERATURE ON THE CHARACTERISTICS OF THE MANGO-FLAVOURED MILK BLENDS AT THE BEGINNING AND END OF THE REFRIGERATOR TEMPERATURE STORAGE (40-45°F).

Characteristics of the mango-milk blends	Storage	
	Beginning	End
5-Non-Stabilized Mango-Milk Blends (Avg. Shelf-life=72-216 hr)		
°Brix	16·000	16·500-18·750
pH	6·075- 6·975	4·950- 5·875
Acidity as lactic acid(%w/v)	0·136- 0·192	0·214- 0·315
Total solids (%w/w)	15·992-16·057	15·980-16·134
Vit C(mg/100 ml)	0·099- 0·445	0·082- 0·235
3-Pectin-stabilized Mango-Milk Blends (Avg. Shelf-life=48-96 hr)		
°Brix	16·000	16·375-16·975
pH	6·250- 6·350	5·600- 5·900
Acidity as lactic acid(%w/v)	0·162- 0·178	0·212- 0·259
Total solids (%w/w)	16·007-16·000	15·995-16·026
Vit C(mg/100 ml)	0·104- 0·302	0·085- 0·246
8-Gelatin-Stabilized Mango-Milk Blends (Avg. Shelf-life=48-348 hr)		
°Brix	16·000	16·500-19·750
pH	6·475- 6·750	5·250- 6·125
Acidity as lactic acid(%w/v)	0·083- 0·155	0·189- 0·304
Tota solids (%w/w)	15·968-16·041	15·989-16·192
Vit C(mg/100 ml)	0·099- 0·363	0·047- 0·230

The effect of temperature on the storage life of the blends was quite apparent. At room temperature (80–95°F) only 2 blends, K-3 and H-2 had a shelf life of 25.5 and 25.0 hr respectively while the shelf life of remaining blends varied from 13.0 to 21.5 hr. But at the refrigerator temperature (40–45°F), 6 blends viz, S-5, S-4, K-1, K-3, K-2 and H-2 survived for 132, 216, 228, 240, 336 and 348 hr respectively while the stability of the rest varied between 48–96 hr (Table 3).

Tables 4 and 5 respectively show the effect of time and temperature on the characteristics of the mango-flavoured milk blends at the beginning and the end of room (80–95°F) and refrigerator (40–45°F) temperatures storage.

The cumulative effect of temperature and storage on the physicochemical characteristics and organoleptic quality of the blends was also observed and recorded as under: (a) °Brix of all blends increased with storage life. (b) Per cent total solids were almost constant. However in some cases the values of the total solids fell below the minimum °Brix which apparently may be due to some experimental error. (c) Browning in the shades of the visual colours of the blends was observed on storage. (d) pH decreased more rapidly at room storage than at the refrigerator storage in all blends except the gelatin-stabilized blends where the rate of decrease was steady. (e) Acidity of all blends except the gelatin-stabilized increased tremendously at room storage than at the refrigerator storage. (f) Vitamin C decreased in all blends fairly uniformly irrespective of the composition of the blends. (g) Organoleptically, all blends scored between good to fair grades during the first 12 hr at room temperature but afterwards rapidly degraded from poor to very poor grade. At refrigerator storage, during the first 24 hr all blends improved their organoleptic scores but afterwards began to deteriorate in quality gradually up to the 144 hr interval, after which the fluctuations in quality remained constant within the 'fair' grade till spoilage.

Four Formulations are being recommended as recipe, i.e. H-2, K-2, D-1 and G-1. H-2 and K-2 blends can be stored successfully for a period of a fortnight or more when stored at 35–40°F. D-1 and G-1 excell all the remaining blends in their overall better organoleptic quality but they lack in good storage life.

References

1. *Standard Methods for the Examination of Dairy Products*: *Microbiological and Chemical* (Am. Pub. Health Assoc., New York, 1968), p. 399.
2. M.B. Bhatti, A. Sattar and M.R. Chaudhary, Agr. Pakistan, **16,** 485 (1965).
3. V.L.S. Charley, J.Soc. Dairy Technol, **8,** 485 (1955); Chem. Abstr., **49,** 14218.
4. M. Fareed, *Methods of Vitamin Assay* (Interscience, New York, 1966), p.294.
5. H. Gorecka, D. Maczynska, E. Rembowski, J. Lipowski and J. Zaleski, Prace Inst. Lab. Badawczych, Przemyslu Spozywcsego, **16,** 5 (1966); Biol Abstr., **48,** 7336.
6. I.I. Hendrick, S.H. Zchanderl and W.B. Hunter, Am. Dairy Rev., **30,** 42, 76 (1968); Dairy Sci. Abstr.; **30,** 370.
7. W. Horzitz, *Official Methods of Analysis* (AOAC, Washington, 1965), p.141, 415.
8. J.E. Hughes, *Fruit Juice-Milk Beverage*, U.S. Patent 28, 53, 386 (1958), Chem. Abstr., **53,** 1585.

9. J.K. Krum, Food Eng., **27,** 74 (1955).
10. A. Maerz and M.R. Paul, *A Dectionary of Colour* (McGraw Hill, New York, 1950), p.27.
11. A. Monzini and U. Bogntti, Atti. III.Simp. Intern. Zootec., **1968,** 833 (1968); Dairy Sci. Abstr., **31,** 499.
12. E. Rembowski, D. Maczynska, J. Zaleski and H. Gorecka; Przem. Ferment. Rolny, **10,** 488, (1966); Dairy Sci. Abstr., **29,** 546.
13. J.A. Ruck, *Chemical Methods for Analysis of Fruit and Vegetable Products,* Publication No. 1154, (Canada Department of Agriculture, Ottawa, 1963), p. 21.
14. A. Sattar and M.B. Bhatti, Agri. Pakistan, **14,** 429, (1963).
15. C.H. Shih, J. Agr. For. Taichung, **15,** 265, Dairy Sci. Abstr., **31,** 243.
16. W.B. Smith, *Sterilized Liquid Milk Shake Mix.* U.S. Patent 33,85,714 (1968); Chem. Abstr., **69,** 3238.

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UTILIZATION OF CELLULOSIC WASTES

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Abstract. Reduction in the particle size of bagasse and rice husk from 60–120 mesh resulted in an increase in the release of sugars from 0.6–6.0 mg/g. Heating of the samples at 125°C for 30–40 min released maximum amount of sugar 15.8 and 5.93 mg/g from bagasse and rice husk respectively. Soaking in 2N HCl and 2% NaOH for 1 hr at ambient temperature released 2.8 and 1.85 mg/g sugar from bagasse and rice husk respectively. Bagasse samples previously heated at 125°C for 40 min when refluxed with 2N HCl for 40 min gave maximum release of sugar, i.e. 282.33 mg/g.

About 2.5 million tons of cellulosic wastes, such as bagasse (954,609 tons), wheat straw (6,605,000 tons), rice straw (13,165,000 tons), and rice husk (4,388,000 tons) are available in Pakistan. Though wheat bran, beet pulp, cotton linters and paper wastes form a sizeable amount of the wastes but unfortunately the figures are not available presently. These wastes contain from 50 to 70% of carbohydrates which cannot be degraded easily into metabolizable sugars by animals. Recently, a large number of rumen and soil microorganisms have been found to possess the ability of degrading native cellulose [1–5] *in vitro*, and converting it into a wide range of products from hydrocarbons to carbohydrates.

Whitaker[7] reported that enzymic degradation of cellulose was by random hydrolysis; addition of urea either inhibited or stimulated this activity depending upon the pH of the medium.[6,7] An increase in the susceptibility of cellulose to the action of the enzymes was observed with modification of cellulose by physical or chemical means.[8–12] Present studies were aimed at establishing optimum conditions for biodegrading bagasse and rice husk into easily disgestible products.

Materials and Methods

Sample Preparation. Bagasse or rice husk was ground and samples of different particle sizes were prepared by sieving through 22, 60, 80, 100 and 120 mesh sieves. A ball mill was used for fine grinding.

Estimation of Sugar. One g sample of cellulosic material was extracted with 100 ml water or 2% NaOH or 2N HCl. Alkaline and acidic extracts were neutralized and reducing sugars present in the extract were determined by Fujita and Iwatake method.[13]

Cellulose Determination. Cellulosic sample (½–2 g) was taken in 100 ml water and was homogenized in a Waring blender for 20 min. Ten ml homogenized sample was taken for estimation of cellulose.[14]

Estimation of Starch. Starch present in 0.5 g bagasse or rice husk was estimated by A.O.A.C. method.[15]

Results and Discussion

Effect of Reduction in the Particle Size on the Release of Sugars. An increase in the release of sugar from 0.6 mg/g to 6.0 mg/g was noted when particle size of bagasse sample was reduced from 60 mesh to 120 mesh. This shows that reduction in particle size increased the surface area which improved extractability of sugars. Maximum sugar (6.0 mg/g) was releasd when particle size was 120 mesh. 5–8% increase in sugar was also observed by Ghose and Kostick[16] when particle size of Solka Flock was reduced from 149 to 4.6 μ.

Effect of Dry Heat on the Release of Sugars.. The release of sugar increased from 8.75 to 15.2 mg/g with an increase in time up to 60 min when the samples were heated at 100°C. Increase in temperature from 100 to 125°C resulted in a quicker release of sugar. Maximum sugar was released (15.8 mg/g) when the samples were kept at 125°C for 40 min. Further heating for 10 min did not show any increase in the release of sugar. A decrease in sugar content from 15.8 to 15.0 mg/g was noticed when the sample was heated for 60 min. Similar results were obtained when the samples were kept at 150° and 175°C.

An increase in sugar content from 0.81 to 5.93 mg/g occurred when rice husk samples were heated for 30 min at 100°C. Increase in heating time up to 45 min did not improve the release of sugar. Further increase in heating time up to 60 min resulted in a decrease (i.e. 0.56 mg/g) in the release of sugar. An increase in temperature (from 100° to 125° and 150°C) and time (30–60 min), showed a gradual decrease in sugar content (from 5.93 to 3.43 mg/g). Maximum sugar (5.93 mg/g) was released when a rice husk sample was heated for 30 min at 100°C.

Increase in sugar contents seems to be due to oxidative changes brought about by heat treatment and changes in crystalline character of the cellulosic material which renders it more susceptible to the action of cellulolytic enzymes. Ground and heated cellulose was considered a better substrate by Ghose[17]; and Katz and Reese,[18] because it showed more susceptibility to cellulolytic enzymes than the unheated cellulose.

Effect of Acid or Alkali on the Release of Sugars. The sugar content decreased from 0.6 to 0.5 mg/g when bagasse samples were soaked in 2% NaOH solution up to 2 hr (Table 1). This decrease seems to be due to the exposure of alkali-treated cellulose to air, making the substrate more resistant to hydrolysis.

TABLE 1. EFFECT OF ALKALI AND ACID ON THE RELEASE OF SUGAR CONTENT IN BAGASSE AND RICE HUSK SAMPLES.

Sample	Particle size	Soaking	Time for treatment	Sugar concn (mg/g)
Bagasse	60 mesh	—	—	0·6
,,	,,	2% NaOH	1 hr	0·52
,,	,,	,,	2 ,,	0·51
,,	,,	2N HCl	1 ,,	2·8
,,	,,	,,	2 ,,	2·9
Rice husk	22 mesh	—	—	0·81
,,	,,	2% NaOH	1 hr	1·85
,,	,,	,,	2 ,,	1·81
,,	,,	2N HCl	1 ,,	0·9
,,	,,	,,	2 ,,	0·92

A decrease in the release of sugar was reported by Reese[19] when the samples were treated with NaOH solution. This was attributed to the changes which occurred during the exposure of alkali-treated cellulose to air. The reduced susceptibility of the residual cellulose may also be due to the removal of more readily hydrolyzed (amorphous) areas by the formation of substituted cellulose derivatives. Sugar content increased from 0.6 to 2.9 mg/g when bagasse sample was soaked in 2N HCl up to 2 hr. This increase may be due to swelling or opening up of the larger molecules (polymeric structure of the cellulose) by depolymerization.

There was no significant increase (0.81–0.9, 0.92 mg/g) in the release of sugar when rice husk samples were soaked in 2N HCl for 1 and 2 hr. An increase in the release of sugar up to 1.04 mg/g was noted when the sample was soaked in 2% NaOH solution for 2 hr. This increase seems to be due to the removal of silica in the form of sodium silicate (as 48.46% loss in weight was observed after alkali treatment) from rice husk which resulted in the formation of easily breakable cellulose molecules.

Effect of Hydrolysis. There was a gradual increase in the release of sugar from 196.25 to 27.5 mg/g when bagasse preheated at 100°C was refluxed for 30–60 min. However, maximum release of sugar (282.33 mg/g) was observed in bagasse preheated at 125°C and refluxed for 40 min. Further increase in refluxing time resulted in a decrease in the release of sugar. Bagasse preheated at 150–175°C also showed the above pattern for the release of sugar.

Release of sugar in preheated rice husk at 100, 125, 150°C varied from 54.07 to 134. 38 mg/g. The increase in preheating temperature 100–125°C and refluxing time 30–60 min showed an increase in the release of sugar. Maximum amount of sugar was released when the sample was heated at 125°C and subsequently refluxed for 60 min. Rice husk preheated at 150°C and subsequently refluxed for 30–60 min did not show any increase in the release of sugar.

A comparison of these results with those obtained by subjecting bagasse and rice husk to dry heat only showed a significant increase in the release of sugar (8.75–282.33, 5.93–134.38 mg/g). The increase in sugar content might be due to the hydrolysis of polysaccharides in HCl, which deeply penetrates into the extended amorphous surface. Ghose[17] also observed an increase in sugar content and interpreted his results in this way when he soaked Solk Floc in 1N HCl.

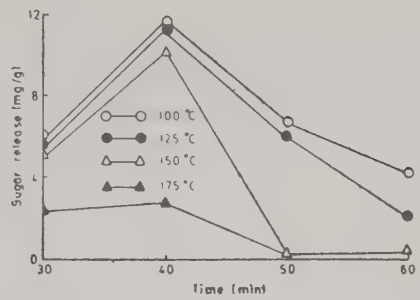

Fig. 1. Effect of dry heat on release of sugar from prerefluxed bagasse.

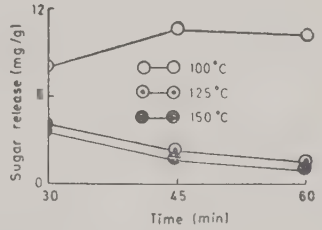

Fig. 2. Effect of dry heat on the release of sugar from prerefluxed rice husk.

Maximum sugar (11.6, 11.8 mg/g) was released when bagasse and rice husk was refluxed for 40 and 45 min respectively prior to heating at 100°C (Figs. 1 and 2). Further increase in refluxing period and heating period and heating temperature caused a decrease in the release of sugar in both the cases. It was observed that the amount of sugar released by refluxing prior to heating of bagasse and rice husk under different conditions of time and temperature was much less than those obtained by heating followed by refluxing. Ghose[17] also preferred preheating while working with Solk Floc. This increase in the extractability of sugar indicated that heat treatment of cellulosic material with modified crystal structure increased its susceptibility towards saccharification.

References

1. H.O.W. Eggins and K. A. Malik, Antonie van Lecuwenhock, J. Microbiol. Serol., **35,** 178 (1969).
2. H.O.W. Eggins, K.A. Malik and R.F. Sharp, Proc. Ist Intern. Biodeterioration Symp., 121, (1968).
3. K.A. Malik and H.O.W. Eggins, Trans, Brit. Mycol. Soc., **54,** 289 (1970).
4. M. Yrmossym, G. J. Weiszfeiler, Maria Somla and J. Hajdu, Proc. Microbiol. Res. Group, Hungarian Acad. Sci., **2,** 97 (1968).
5. K.A. Malik and H.O.W. Eggins, Intern. Biodeterioration Bull., **5,** 163 (1969).
6. D.R. Whitaker, Science, **116,** 90 (1952).
7. P.K. Datta, K.R. Hason and D.R. Whitaker, Biochem. Biophys. Acta., **50,** 113 (1961).
8. G. Halliwell, Biochem. J., **79,** 185 (1961).

9. G. Halliwell, Biochem. J., **85,** 67 (1962).
10. R.G.H. Siu, *Microbial Decomposition of Cellulose with Special Reference to Cotton Textile* (Reinhold, New York, 1951), p. 159
11. E.T. Reese, R.G.H. Siu and H.S. Levinsons, J. Bact,. **59,** 485 (1950).
12. T.K. Ghose, Biotech. Bioengg., **11,** 239 (1969).
13. A. Fujita and D.C. Iwatake, Biochem. Z. **2,** 243 (1931).
14. D.M. Updegraff, Anal. Biochem., **32,** 420 (1969).
15. W. G. Pucher, C. S., Leavenworth, and H. B. Vickery, Anal. Chem., **20,** 850 (1948).
16. T.K. Ghose and J.A. Kostick, Biotech. Bioengg., **12,** 921 (1970).
17. T.K. Ghose, Biotech. Bioengg., **11,** 239 (1969).
18. M. Katz, and E.T. Reese, Appl. Microbiol., **16,** 419 (1968).
19. E. T. Reese, Appl. Microbiol., **4,** 39 (1956).

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Short Communication

EFFECT OF EXTRACTION TECHNIQUES ON THE EXTRACTION OF PROTEIN FROM TRIFOLIUM ALEXANDRINUM

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(Received October 15, 1974; revised September 25, 1975)

Extraction of proteins from leaves has been studied by various workers.[1–4] Byers and Sturrock[5] made a comparative study on the extractability of proteins from various leaves. Chayen *et al.*[6] described impulse rendering process to separate proteins from plants. More recently Davys and Pirie[7,8] developed a machine to extract leaf proteins on pilot scale. Knuckle *et al.*[9,10] extracted protein from leaves using sugarcane rollers.

In the present studies International Biological Programme (IBP) pulper, crypto meat mincer AB-12-74793 (power operated), hand-operated and power-driven sugarcane crushers were used to disintegerate the leaves of *Trifolium alexandrinum*. Juice was extracted by pressing the pulp with a belt press, IBP press and by hands. Proteinous nitrogen (PN) was precipitated with an equal volume of 20% solution of trichloroacetic acid (TCA) and separated by centrifugation at 300 rev/min. The preciptate was analysed for PN and the supernatent for non-proteinous nitrogen (NPN). Nitrogen was determined by a micro-kjeldahl method using $CuSO_4$–K_2SO_4–SeO_2 (1:9:0.02) mixture.[11]

Results and Discussion

Effect of Extraction Techniques on the Extractability of Proteins. Extraction techniques greatly affected the extractability of proteins. It was maximum (60.2%) when the crop was pulped with IBP pulper and the juice was extracted by hand pressing (Table 1). 57.8% proteins were extracted with IBP press and 51.7% with the belt press. The increase in the extraction of proteins by hand pressing seems to be due the higher pressure applied by hands, which resulted in the maximum extraction of chloroplastic as well as cytoplasmic proteins. The decrease in the extractability in case of IBP press or the belt press seems to be due the formation of fibrous cake which caused lesser filtration and greater reabsorption of the chloroplastic proteins. These results agree with the findings of Davys and Pirie.[12]

Extractability of protein was low when crypto meat mincer, power-operated sugarcane crusher or hand operated sugarcane crusher was used for pulping the crop. In case of cyrpto meat mincer it was 50% with hand pressing, 50% with IBP press 43.6% with the belt press. With power operated sugarcane crusher the extractability was 24% in March with hand pressing, 21.0% with IBP press and 20% with belt press. Similarly in case of hand operated sugarcane crusher the extractability varied from 15.5–16% (Table 1). Low extraction with crypto meat mincer, power-operated or hand operated sugarcane crusher appeared to be due to the incomplete rupturing of the cell, and disintegration of the plant tissues which resulted in retention of chloroplastic proteins by the fibrous matter.

Although the extractability of protein with hand pressing and IBP press was higher than that of belt pressing but pressure applied by hand may vary from person to person. Moreover, it seemed unlikely that this technique would be applied for bulk production of leaf protein concentrate. Further, proteins present in the juice extracted by hand pressing or with IBP press were hydrolysed to a greater extent by the proteolytic enzymes present in it due to greater time consumed during these operations. The hydrolysis of protein resulted in a decrease in the PN which was accompanied by an increase in the NPN contents of the juice (Table 2). Hydrolysis of proteins by proteolytic enzymes has also been reported.[13–18]

Extractability of protein also varied considerably. with temperature. Maximum extractability, i.e. 60% was observed during winter (January) which decreased to 35% during May (Fig. 1). The decrease in the extraction rate of protein during these days appeared to be due to decrease in moisture con-

Table 1. Effect of Extraction Techniques on the Extraction of Protein from *Trifolium alexandrinum*.

Pulping machines	Extractability of protein (%)*					
	January			March		
	Belt pressing	Hand pressing	IBP pressing	Belt pressing	Hand pressing	IBP pressing
IBP pulper	51·7	60·2	57·8	44·73	50·50	50·00
Crypto meat mincer	43·6	50·0	50·0	34·00	44·10	40·00
Power operated sugarcane crusher	—	—	—	20·00	24·00	21·00
Hand-operated sugarcane crusher	—	—	—	15·50	16·00	15·50

*Extracted protein-N expressed as a% of the total N of the leaf.

TABLE 2. EFFECT OF EXTRACTION TECHNIQUES ON PN AND NPN OF JUICE OF *Trifolium alexandrinum*.

Pulping machines	Percentage of Protenous Nitrogen (PN) and Non-Protenous Nitrogen (NPN)											
	January						March					
	Belt pressing		Hand pressing		IBP pressing		Belt pressing		Hand pressing		IBP pressing	
	PN	NPN	PN	NPN	PN	NPN	PN	NPN	NP	NPN	PN	NPN
IBP pulper	90·00	10·0	89·0	11·0	87·1	12·9	89·0	11·0	87·0	12·0	85·1	12·9
Crypto meat mincer	87·0	13·0	86·1	13·9	85·0	15·0	84·8	15·0	84·5	15·5	83·3	16·7
Power operated sugarcane crusher	—	—	—	—	—	—	83·8	16·2	81·0	79·0	79·0	21.0
Hand operated sugarcane crusher	—	—	—	—	—	—	80·7	19·3	71·6	28·4	70·0	30·0

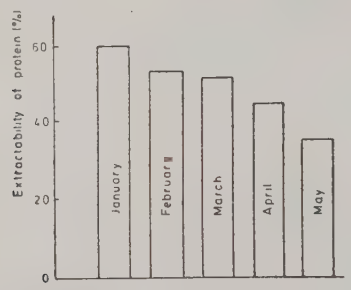

Fig 1. Variation in the exrtactabilily of protein (using IBP pulper and hand pressing)

tents of the plant which was 91.6% in January, 90.6% in February, 90% in March, 89.7% in April and 83.6% in May. Similar observations have been made by Shah *et al.*[19] during studies on the extractability of proteins from various grasses.

References

1. A.C. Chibnall, E.J. Miller, D.H. Hall and R.G. Westall, Biochem. J., **27,** 1879 (1933).
2. N.W. Pirie, Chem. Ind., p. 61 (1942).
3. N.W. Pirie, Proc. Nutr. Soc., **15,** 154 (1956).
4. R.E. Slade, D.J. Branscombe, and J.C., McGowan, Chem. Ind., 194 (1945).
5. M. Byers and J.W. Sturock, J. Sci. Food Agr., **16,** 341 (1965).
6. I.H. Chayen, R.H. Smith, G.R., Tristram, D. Thirkell, and T. Webb, J.Sci. Food Agr., **12,** 502 (1961).
7. M.N.G. Davys and N.W. Pirie, Biotech. Bioengng., **11,** 571 (1969).
8. M.N. G. Davys, N.W. Pirie and G. Street, Biotech. Bioeng, **11,** 528 (1969).
9. B.E. Knuckles, R. Rolend, Sepencer M.E. Lazar, E.M. Bickoff and G. O. Kohler, J. Agr. Food Chem., **18,** 1086 (1970)
10. B. E. Knuckles, E.M. Bichoff and G. O. Kohler, J. Agr. Food Chem., **20,** 1055 (1970).
11. R. Markhem, Biochem.J., **36,** 790 (1942).
12. M.N.G. Davys and N.W. Pirie, J. Agr. Eng. Res., **10,** 142 (1965)
13. O. Loew, U.S.Dep. Agr. Rep., No. 65 (1907).
14. M.N. Tracey, Biochem.J., **42,** 281 (1948).
15. H.T. Macpherson, J.Sci. Food Agr., **3,** 362 (1952).
16. C.J. Brady, J.Sci. Food Agr., **11,** 276 (1960).
17. G.J. Brady, Biochem. J., **78,** 631 (1961).
18. N. Singh, J.Sci. Food Agr., **13,** 325 (1962).
19. F. H. Shah, Ann. Res. Rept., PL–480 Scheme, Investigstion on the Preparation and Composition of Protein Concentrate From Grasses, PCSIR Laboratories, Lahore (1973).

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BOOK NOTICES

Energy: Use, Conservation and Supply. A Science Compendium, edited by Philip H. Abelson. 150 pp. American Association for the Advancement of Science, Washington, 1974.

The energy crisis of 1974 had a profound effect on all facets of life the world over. Scientists particularly, who are deeply involved in interpreting the phenomena relating to all sources of energy as well as analysing different methods of production and utilisation of various forms of energy felt its impact more than others. SCIENCE, the weekly journal of the American Association for the Advancement of Science published some excellent articles covering various aspects of energy spectrum in the context of the crisis and brought out a special issue on energy. The Compendium is based on selection from these articles.

The material, naturally, largely is devoted to the American scene though, of course, the purely scientific and technological aspects of the discussions are divorced from local overtones and have a global hearing. Nevertheless, one gets the feeling after going through the material that it was written mostly for the consumption of the American public. The section "Developing Technology" though is a more detached view of theings and the technical material therein can be kept as a reference by every student of science.

The Compendium is but a unique book with such a fund of knowledge about energy, packed with data bearing on all its gamuts. No single book carrying so much authentic material on gas, coal, oil, nuclear power, solar energy, geothermal potential, wind power and hydrogen potential may possibly be available within one cover. Even food production in relation to energy crisis has been discussed. Conservation aspect of energy looms large practically everywhere, as, no doubt, the American citizen has been in the habit of using energy profusely and rather indiscriminately. The article on Energy Conservation and that on Conservation in industry are such that they can reach a wider class of readers including an average citizen in the developing countries.

The article on oil shale is particularly interesting as going through it makes one feel knowledgable about a new energy source with vast possibilities, though the pollution aspect in the production of oil shale produces a damping effect on the otherwise refreshing picture.

The article "Energy Choices that Europe Faces" is with a difference. It depicts and analyses the problems of Europe under the shadow of the energy crisis and which quite often dovetails into what is faced by developing countires as well.

The presentation of each article is different from the other in the very nature of things and that makes reading much more interesting than of a book of 150 pages by a single author. The various shades seen, typical of each writer, make it a colourful reading material that leaves the reader much wiser about various aspects of energy in the world.

The price is not mentioned in the publication. May be, it is meant for distribution to the members of the American Association for the Advancement of Science only. It will be a pity if that is so, since every Library should own the publication even if it is not a subscriber to Science.

Azim Kidwai

Major Research in Upland Rice. pp. 255. International Rice Research Institute, Los Bamos, Phillipines, 1975.

Upland rice has been defined as rice grown on flat or sloping fields that are not bunded, that were prepared and sown under dry conditions before the advent of rains and the crop depends entirely on rainfall for the moisture it needs. It is obvious from the above definition that the yield of the crop would be highly variable from place to place and from year to year and further will be much lower than that of lowland rice which thrives on irrigation. The yield can however, be improved by breeding new high yielding varieties and through the improvement of cultural practices to suit the soil, climate and social conditions. It is contended that much of the possible future expansion in rice area will probably be in upland rice as the low land areas where irrigation can be applied have been fully exploited all over the world. Form this observation stems the importance of the research carried out on upland rice.

Most upland rice varieties are tall, lodging susceptible, low-tillering and low-yielding types. In Asia the biggest area under upland rices occur in Indonesia, Bangladesh and the Phillipines. In Africa about 75 per cent of the total rice area is under upland varieties and about 65 per cent of the rice crop of Latin America is of the upland variety.

Rainfall and its distribution are the most important element of environment. Most of the upland rice is grown in the monsoon belt of Asia. A precipitation of 100 mm per month during growth is quite satisfactory.

There are detailed accounts of the optimum daily sunlight, photoperiod, soil texture and quality, fertiliser application and plant protection measures, but of particular interest are the details of plant breeding and plant physiological work which has so far been carried out in the Phillipines and elsewhere.

There are eight chapters which include 76 sections covering the entire spectrum of studies so far carried out on upland rice. This publication is therefore, very useful as a reference book for workers on the rice crop. Copious reference are made to the research publications and at the end 292 references are citited.

The language used is simple and exact which is easily comprehensible. The bulletin is an extremely useful book of reference for the rice research workers even of Pakistan where only low land rice is grown as its perusal widens the horizon of the reader.

Muhammad Afzal

Changing Priorities for Government R & D. An experimental study of trends in the objective of Government R & D funding in OECD member countries, 1961–72. 322 pp. Organization for Economic Cooperation and Development, Paris, 1975. $13.00; £5.20; f52,00.

Contents: General introduction; Setting national priorities for Government R & D; The problems of comparing national priorities for Government funded R & D; The Comparison; The outlook for Government R & D funding; Government R & D funding by objective.

Science in Flux. Joseph Agassi. 530 pp. D. Reidel, Dordrecht, Holland, 1975. Price: cloth bond, $48.00; paper back $24.00.

This volume, dedicated to Sir Karl Popper, by one of his foremost disciples and his acknowledged severest critic, offers in integrated view and a new demarcation of metaphysics, science, and technology. The accent is on the social character of science and on the existence of scientific revolutions, large and small. In the tradition of critical rationalism, the author comments on the giants of our philosophical and scientific heritage, as well as on the leading thinkers of our century. The author's papers on the role metaphysics plays in the formation of scientific research programs, as well as his papers on the philosophy of technology, have started a widespread discussion in the professional literature. The author offers a dynamic view which stresses that the conflicts between science and metaphysics, between the scientific and the technological attitude towards positive evidence, are the leavn of scientific progress. The author's clear and engaging style and his provocative views and arguments, promise enjoyable hours of reading. In addition an avant-grade comprehensive view of science should emerge from this collection of essays.

The Cultural Context of Medieval Learning. edited by J.E. Murdoch and E.D. Sylla 540 pp. D. Reidel, Dordrecht, Holland 1975. Price: cloth bond £49.00; paper back $28.00.

The comparative historical sociology of science—fo lack of an adequate and more economical name—ir as many-sided as it is many-syllabled. This book sxhibits the confluence not only of historical and eociological contexts of science, but also of the concrete philosophical, theological, political, and legal contexts which investigation of a particular comparative case study required. The choice of the particular period, its rationale, and the fruitfulness of the results, is well described in the Introduction. All scholars will understand our admiration for the skill, intelligence, and care with which Murdoch and Sylla have brought the prepared materials and the reported discussions into this beautifully integrated and lucidly articulated book. It was Murdoch who took on the demanding task of organizing the intellectual spectrum of interests and competences that resulted in as original and rich an exploration as the conference afforded and this book presents. It shows again how wide a perimeter of conceptual issues the history and philosophy of science can generate, when they are conceived in the fuller contexts of their origin, development, and social milieu. Appreciating as we do the editors' note of caution about the treatment of social, political, and economic factors in the analysis of medieval thought and culture, it also becomes clear to us that a rigorous and careful investigation of the relations between material culture and both conceptual activities and institutional forces is desired in order to enlarge and deepen these studies. Need we add that we hope to see other periods treated with equal care and penetration? We also expect that philosophers of modern science will find here insights into their own conceptual problems, as well as some historical roots of these problems.

The Iron and Steel Industry in the Developing Countries. Report of the Third International Symposium on the Iron and Steel Industry held at Brasilia, Brazil, 14-21 October, 1973. 99 pp. United Nations, New York, 1974. U.S. $6.00.

Contents: Introduction; Conclusions and recommendations; Organization of the Symposium; International cooperation in the establishment and expansion of iron and steel industries in developing countries; Environmental aspects of iron and steel industries in developing countries; The planning and financing of iron and steel projects in developing countries; Economic and marketing aspects of the iron and steel industries in developing countries; Technological developments in the iron and steel industry: raw materials and ironmaking in the blast Furnace; Technological developments in the iron and steel industry: direct reduction; Technological developments in the iron and steel industry: steelma king and casting; Technological developments in the iron and steel industry: rolling and finishing processes; The creation and transfer of matallurgical know-how to and between developing countries; Problems of management and manpower in the iron and steel industries of developing countries; Potential technical assistance projects for the iron and steel industries of developing countries; list of papers prepared for the Symposium; List of plants visited.

Irrigation, Drainage and Salinity. 510 pp. Hutchinson/FAO/UNESCO, London, 1973. Price: $10.00; $30; FF 120.00.

Throughout history, irrigation in arid zones has often led to soil salinization, thereby creating a problem which has faced man for centuries. With the increase of the world's population and the rapid development of irrigated areas, this question becomes more and more urgent: not only have unproductive lands to be put under cultivation but also the fertility of irrigated areas has to be maintained.

The experience acquired throughout the centuries and the considerable amount of research which has been carried out on irrigation and soil salinization have produced a great vareity of data. In their work, Unesco, within the framework of its Arid Zone Major Project, and FAO in its activities on Land and Water Development, have already made constructive efforts in the collection, synthesis, and dissemination of such knowledge. However, man is often hesitant when attempting to select the best method for the improvement of existing irrigation schemes which are being spoiled by waterlogging and salinity and for the reclamation of virgin lands where these hazards may arise. Such a situation is obviously detrimental to newly developing and emerging countries where the shortage of experienced staff makes it imperative to provide simple and clear guidance on the procedures to be followed. During a joint meeting of FAO and Unesco Secretariates in 1960, it was proposed to carry out a world-wide survey of the knowledge available and eventually to publish the information obtained. The aims of this publication would be:

(a) To provide a summary of modern scientific concepts in a form convenient for use by administrators, engineers, agronomists, hydrotechnicians, soil scientists, and other specialistsd ealing with irrigation and drainage methods and practices in relation to salinity and alkalinity of arid lands.

(b) To review procedures for forecasting water requirements of crops and the irrigation and drainage needs for irrigation projects under various conditions, giving particular attention to the prevention of salinity and alkalinity.

(c) To discuss irrigation and drainage systems and management problems in relation to salinity and alkalinity.

(d) To assemble data required by the specialists concerned in the design and operation of irrigation and drainage works in the arid zone.

In other words, it was proposed to prepare a sourcebook which would provide (1) a synthesis of modern scientific knowledge, (2) a guide to the practical experience obtained by leading countries in the field of irrigation and drainage methods in relation to salinity and alkalinity of arid lands and (3) generalizations useful in the solution of practical problems in this field.

This proposal was adopted by the General Conferences of Unesco in November 1960 and of FAO in November 1961. To attain the proposed goal, a special procedure was needed in order to provide for general consultation and exchange of opinions on the subject. It was pointed out at the beginning that such an integration of knowledge on an international basis would required numerous contacts in order to make sure that all opinions were duly taken into consideration; moreover, this was the first time that such a work had been undertaken on a world-wide scale. At the first meeting in Rome in 1960, it was decided that a group of three editors should be appointedjointly by FAO and Unesco and that authors and co-authors for the different chapters should be selected by mutual agreement from among the most experienced specialists working personally on the problems under consideration. In addition, contributors selected from member countries of either organization would be invited to co-operate. The source-book would thus be a synthesis of all the information gathered and no effort would be spared in order to ensure that the book gave a complete review of the knowledge available in the world, making clear the points of agreement and indicating subjects where research is needed.

Accordingly, FAO and Unesco asked Prof. V.A. Kovda, Prof. R.M. Hagan and Dr. C. Van den Berg to act as editors. They generously accepted this responsibility and nominated Prof. Kovda as co-ordinating editor. In addition, M. de Forges, as technical secretary, agreed to assist the editors in their work.

A detailed outline was drawn up by the Editorial Board and during the preparation more than 100 specialists from 20 countries were kept informed of the work. Some of these specialists were requested to submit manuscripts as a basis for the drafting of given chapters. Others were invited to prepare papers on specific points they were studying. The material was then reproduced as 'working documents' and circulated among specialists, who then submitted comments and complementary notes.

The Editorial Board held several working sessions in order to examine this material. These meetings took place in various countries: France, the Netherlands, Pakistan, USA and USSR. Thus, the editors have had an opportunity to establish personal contact with specialist belonging to various scientific disciplines.

A provisional draft was distributed to scientific and technical circles and the ensuring comments and suggestions were considered by the Secretariat in the preparation of the text. The text is a synthesis of the considerable amount of material collected and it is obvious that some deleting has been unavoidable.

Statistical Inference. S. D. Silvey. 192 pp.! Chapman and Hall, London, 1975. Price: £2.30.

Nuclear Techniques and Mineral Resources. 546 pp. International Atomic Energy Agency, Vienna, 1969. Price: $13.00.

Contents. Our geophysics and processing; Uranium exploration, Radio-isotope fluorescence; Oil fields geophysics, Activation analysis.

Catalogue-1974. 195 pp. International Atomic Energy Agency, Vienna, 1974.

Beginner's Guide to Transistors. J. A. Reddihough and I. R. Sinc air. 162 pp. Newnes—Butterworths, London, 1975. Price: £1.80.

Schizophrenia: A Multinational Study. A Summary of the Initial Evaluation Phase of the International Pilot Study of Schizophrenia. 150 pp. World Health Organization, Geneva, 1975. Price: Sw.fr.16.

Laboratory Techniques in Brucellosis. G. G. Alton, Lois M. Jones and D. E. Pietz. 163 pp. World Health Organization, Geneva, 1975. Price: Sw.fr.35.

Hidden Harvest. David Spurgeon. 36 pp. International Development Centre, Ottawa, 1976.

Corrigendum

*The Stability Constants of Co(II) and pyridine-2-azo-*p*-dimethyl aniline 1:1 Complex in Mixed Solvents* by Shabbir A. Zubairi, Pakistan J. Sci. Ind. Res., **18,** page 213 (1975), column 1, para 1, and the following after.....water bond strength (1st line).

The $\triangle H$ values go through a minimum around 3.31 mole per cent of methanol and a corresponding maxima is observed in $\triangle S$ values at the same mole fraction, indicating an inversion of cosolvent effect around this concentration. [4, 11, 16, 18] Since the dffiusion controlled ion-pair formation step is independent of dielectric constant for electrically neutral ligands, the changes in K and in enthalpy and entropy must originate from steps 2 and 3. The order imposed by the cosolvent on bulk solvent presumably increases ease of removal of water from primary coordination sphere.[17] This is reflected in more negative enthalpy and more positive entropy.